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Therapeutic monoclonal antibodies in ophthalmology

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A B S T R A C T

Monoclonal antibodies (mAbs) can be used therapeutically by binding to molecular targets with high specificity. Therefore, they have excellent therapeutic applications in ophthalmology. This manuscript presents four aspects of the therapeutic use of mAbs in ophthalmology: the scientific rationale, the unique characteristics of selected mAbs, the current state-of-the-art application, and relevant therapeutic mAbs for future applications in ophthalmology. We identified in the literature various single-agent therapies that inhibit the following targets: tumor necrosis factor (TNF), epithelial growth factor receptor, vascular endothelial growth factor (VEGF) receptor, basic fibroblast growth factor receptor, platelet-derived growth factor, and cluster of differentiation antigens. The roles of all biochemical targets in ocular diseases were evaluated. Current and future mAbs against various cytokines were assessed for the treatment of ocular diseases. The medical literature showed the clinical benefits of mAbs for treating angiogenic and inflammatory ocular diseases. Two anti-VEGF mAbs, bevacizumab and ranibizumab, and three anti-TNF agents, infliximab, etanercept, and adalimumab, control ocular neovascularization and intraocular inflammation. Other mAbs such as rituximab, daclizumab, efalizumab, and alemtuzumab showed positive results in animal and early clinical studies and may represent useful adjuvant therapies for ocular lymphoma or ocular inflammation. Ranibizumab is the only FDA-approved therapy; for other mAbs the so-called off-label application remains the standard. Intravenous administration of mAbs has demonstrated acceptable toxicity profiles, while intraocular injection may decrease the chances of systemic complications and increase the amount of drug available to the retina and choroid. In conclusion, effective clinical use of mAbs in ophthalmology is more commonly seen in the field of angiogenic vitreoretinal and autoimmune inflammatory diseases. The challenge for the future is combining biologic therapies to improve the quality and duration of responses while diminishing side effects. The role of mAbs within ophthalmic treatments will be defined according to future clinical experience and the results of randomized clinical trials.

Contents

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1. Introduction

Monoclonal antibodies (mAbs) are a class of antibody molecules comprised of one type of immunoglobulin produced from one B-cell clone that recognizes a specific antigenic target (Teillaud et al., 2002). mAb production was introduced as a research tool in 1975 with the advent of hybridoma technology in animals, followed by the development in 1986 of the murine anti-CD3 mAb for therapies (Kohler and Milstein, 1975; Goldstein et al., 1986). Rejection of these murine mAbs by the human immune system was a barrier to human use, but advances in biotechnology reduced this problem with the production of high-quality chimeric, humanized, or completely human mAbs (Fig. 1) (Jones et al., 1986; Lonberg, 2005; Vitetta and Ghetie, 2006). These advances led to the development of excellent therapeutic agents for neoplastic, rheumatologic, and infectious diseases, with a substantial impact on improvement in human health (Reichert, 2001; Waldmann, 1991).

In ophthalmology, therapeutic mAbs have been introduced recently to treat inflammatory and angiogenic diseases (Fig. 2) (Avery et al., 2006; Bashshur et al., 2006; Hale and Lightman, 2006; Joseph et al., 2003; Lindstedt et al., 2005). The rationale for mAb application in ophthalmology also is based on a recent understanding of the molecular biology of various ocular diseases. In inflammatory ocular conditions, cytokine tumor necrosis factor alpha (TNF-α, or TNF-A) participates in the pathogenesis of autoimmune ocular inflammatory diseases, e.g., uveitis, and chimeric anti-TNF-α mAb infliximab and the newer adalimumab control uveitis and other inflammatory eye diseases (Dick et al., 2004; Singh and Rai, 2001). Regarding ocular angiogenesis, cytokine vascular endothelial growth factor (VEGF) participates actively in new vessel formation in various neovascular conditions including diabetic retinopathy (DR) and age-related macular degeneration (AMD) (Duh and Aiello, 1999; Miller, 1997). Recently, full-length mAb anti-VEGF bevacizumab (Avastin, Genentech, South San Francisco, CA) and VEGF-fragment ranibizumab (rhuFabV2, Lucentis, Genentech, South San Francisco, CA) have been reported to promote marked regression of intraocular neovascularization in experimental and clinical studies (Kaiser, 2006; Campochiaro, 2004). In the current manuscript, we review the advantages, limitations, efficacy, and risks of mAb application in ophthalmology with emphasis on ocular angiogenesis and inflammation. A list of mAbs used in ophthalmology is summarized in Table 1. Although the role of cytokines may overlap with the pathophysiology of human ocular disease, this manuscript focused on each cytokine involved in ocular diseases to provide better comprehension.

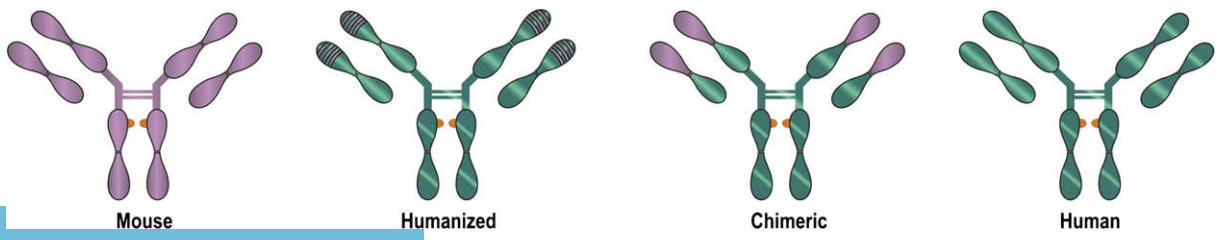

Fig. 1. Schematic description of the four main types of mAbs composition for therapeutics. Purple: mouse sequences; green: human sequences; orange circle: areas of glycosilation.

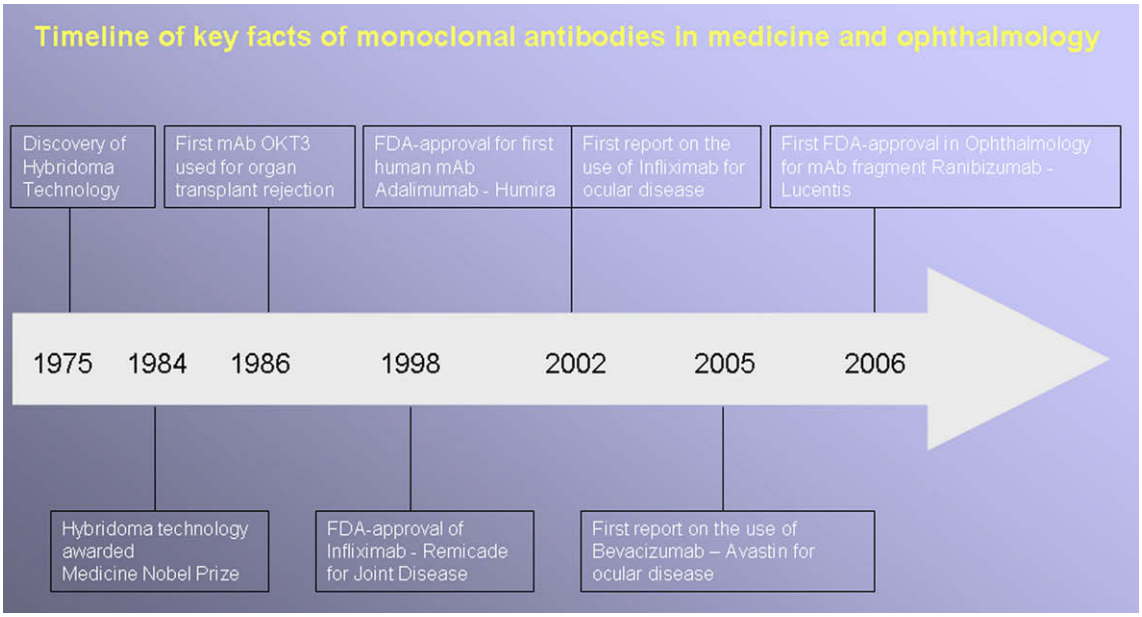

Fig. 2. Timeline of key facts of therapeutic monoclonal antibodies in medicine and ophthalmology. The anti-TNF has been initially applied as endovenous infusions for uveitis, followed by anti-VEGF approaches.

2. Antibody structure and function

Antibodies, Y-shaped glycoproteins with molecular weights of approximately 150 kDa, consist of four polypeptides, two light chains and two heavy chains; heavy chains are constant but light chains have different three-dimensional structures to fit antigens (Foote, 2003; Zack and Scharff, 1983). An antibody is comprised of two antigen-binding fragments "Fabs" with three hypervariable amino acid sequences responsible for antibody specificity and a constant region "Fc" that determines the antibody isotype (IgA, IgD, IgE, IgG, or IgM) and serves as a binding site for complement and leukocytes. Antibodies are secreted by specialized plasma B-lymphocytes as a natural part of the antigen-recognition process of the immune system (Cahalan and Gutman, 2006). Activated lymphocytes proliferate and differentiate into plasma cells to generate polyclonal antibodies; however, mAbs are antibodies produced by one B-lymphocyte clone. mAbs affecting only specificity affect disease-specific molecules due to specificity and spare normal cells. For instance, mAbs may bind to the epitope of antigens for neutralization and enable phagocytosis and complement activation. Therapeutic antibodies can function by blocking specific molecules such as growth factors that prevent receptor binding, as signaling molecules, by blocking essential cellular receptors, by inducing apoptosis, and by recruiting cellular antibody- or inducing complement-dependent cytotoxicity. IgG type antibodies are the first option for the production of mAbs. However, different IgG subtypes may be chosen according to the desired effect. For example, IgG1 is more appropriate when complement-dependent toxicity is necessary, while IgG4 may be applied when antibody action on triggering cascades is unwanted (Johnston, 2006).

3. Overview of production of therapeutic mAbs and fragments

The human immune system response against CD3 muromonab-OKT3, a treatment against transplant rejection, causes strong immunogenic complications that motivate the development of techniques to increase the percentage of human proteins in the components of these therapeutic agents. As a result, the so-called chimeric, humanized, or fully human mAbs were created (D'Alessandro et al., 1989; Lonberg, 2005; Peterson, 2005). Generally, chimeric antibodies contain approximately 33% mouse protein and 67% human protein. However, the murine Fc component is replaced by a human sequence through DNA fusion. DNA segments containing mouse-variable regions are replaced by DNA segments encoding human-constant regions (Kipriyanov and Le Gall, 2004). Although immunogenic complications due to anti-mAbs decreased, they may still occur. Further advances promoted the development of humanized mAbs; for their production, the murine-derived variable regions containing the complementarity-determining regions (CDRs) are

Table 1
Overview of mAbs application in the therapy of ocular diseases.

Drug name	Brand name	Company	Target	Antibody form	Indication in Ophthalmology	FDA-status in ophthalmology
Adalimumab	Humira	Abbott	TNF-A	Fully human	Uveitis and inflammatory diseases	Off-label
Bevacizumab	Avastin	Genentech	VEGF-A	Humanized	Wet age-related macular degeneration, macular edema, and ocular neovascularization	Off-label
Daclizumab	Zenapax	Roche	CD-25	Chimeric	Uveitis	Off-label
Etanercept	Enbrel	Amgen	TNF-A and TNF-B	Recombinant dimeric protein	Uveitis and inflammatory diseases	Off-label
Infliximab	Remicade	Centocor	TNF-A	Chimeric	Uveitis and inflammatory diseases	Off-label
Ranibizumab	Lucentis	Genentech	VEGF-A	Fragment	Wet age-related macular degeneration, macular edema, and ocular neovascularization	Approved
Rituximab	Rituxican	Genentech	CD-20	Chimeric	Lymphoma and inflammatory diseases	Off-label

inserted into the human domain framework resulting in humanized mAbs with about 90% of human proteins. Fully human mAbs with 100% human proteins can be produced by various techniques such as immune donors, phage libraries, or transgenic mice (Weiner, 2006). In 2002, the FDA approved the first human-mAb Humira, and now 15% of clinical mAbs are fully human (Rau, 2002). By July 2007, the FDA had approved 17 therapeutic mAbs, about 230 have been under investigation in clinical trials, with over 1000 in pre-clinical development. These numbers are growing (Reichert and Valge-Archer, 2007). However, the growing importance of mAbs in clinical practice may be hampered by production costs. The total cost of mAb production has been estimated to be about $1 billion with some variation depending on the types of mAbs and targets. Other barriers for extensive use of mAbs include the variability of responses at the target tissues, rejection after recognition by the immune system, and the short duration of the effect.

The mAb fragments represent an attractive alternative to mAbs for therapeutic purposes (Holliger and Hudson, 2005). Fragments are produced by IgG molecules enzymatically digested into smaller functional fragments or by genes encoding the variable/constant domains. In the mAb fragments, various domains of the antibody structure composed of framework and CDRs constitute the "Fv" fragment, whereas the Fab fragment contains the constant domains of heavy and light chains. The advantages of their smaller size include an extended serum half-life and better tissue penetration, although some may have lower affinity. Among the disadvantages of mAb fragments are the lower affinity to target antigens and the much shorter half-life. Techniques in the production of mAbs to improve their efficacy include enhanced antigen-binding affinity through improved the antibody-dependent cytotoxic effector function, enhanced in vivo half-life, and reduced toxicity by conjugation to polyethylene-glycol molecules (so-called PEGylated) (Andersen and Reilly, 2004). These approaches are analogous to the way in which pharmacologists change the chemical characteristics of drugs to optimize the therapeutic options. The improved affinity of mAbs may reduce the dosage needed for therapeutic benefit, increasing the cost-effectiveness of the drug.

While animals may be natural sources of antibodies, one alternative method for mAb construction could be the use of natural transgenic plants. One advantage of plant cells is their flexibility. Up to 10 kg of recombinant antibodies per acre can be obtained from natural sources. Another factor favoring the use of plants rather than animals to produce antibodies is that the former are devoid of human pathogens. However, mAbs extracted from plants (plantibodies) have N-glycans that differ from those secreted by mammalian cells, which may, in theory, promote undesirable side effects in patients.

4. Use of anti-TNF mAbs in ocular disease

Ophthalmologists have initiated the off-label use of mAbs to treat ocular inflammatory diseases for a few reasons: the limited clinical success and partial failure of steroid and immunosuppressant drugs to control ocular inflammation, the high rate of side effects caused by steroid agents, the recognition of molecular cytokines including TNF in ocular inflammatory disease with in vitro assays and animal models, and the previous clinical success of anti-TNF mAbs in controlling systemic inflammatory diseases (Okada, 2005; Tracey et al., 2008). The initial success of anti-TNF mAbs led ophthalmologists to use this therapy in ocular inflammatory diseases, especially for severe and refractory uveitis.

4.1. TNF-α: structure and biology in humans

TNF refers to a family of 19 multifunctional signaling cytokines with important functions in inflammation and apoptosis (McDermott, 2001). The subtype TNF-α is a 17-kDA protein with 185 amino acids, whereas the TNF-B or TNF-β (lymphotoxin) is an 18-kDa glycoprotein with 171 amino acids that forms a homotrimer structure (Hehlgans and Pfeffer, 2005). The biologic activities of TNF are performed through TNF-receptors; the receptor TNFR-1 or p55 is the soluble TNF-receptor expressed on most cell types, whereas TNFR-2 or p75 represents the membrane-associated TNF-receptor, which is usually restricted to some cell types and its expression should be induced (Wajant et al., 2001). Both receptors are transmembrane glycoproteins with extracellular domains sharing similar structures. However, their intracellular domains are distinct; only TNFR1 presents a "death domain," important in the process of TNF-A-mediated cellular apoptosis. Therefore, TNFR1 may mediate most proapoptotic and inflammatory signaling pathways, whereas TNFR-2 may regulate cell growth and proliferation. Biologic TNF-α stimulation may result in leukocyte mobilization/activation, enhanced macrophage-cell killing, and altered vascular endothelial permeability (Chen and Goeddel, 2002). Nevertheless, the superfamily of TNF proteins and their receptors may interact with "double-edged sword" functions: while in some circumstances they may inhibit cellular immune response, in others a proinflammatory reaction may occur.

Immunolocalization of TNF in ocular tissues provides a basis for understanding cytokine function in the normal intraocular environment. In normal mouse eyes, in situ hybridization has shown the presence of both TNFR1 and TNFR-2 receptors within the corneal endothelium, iris, ciliary body, choroid, optic nerve sheath, and vitreoretinal interface. One study reported a signal only for TNFR-2 in the retinal ganglion cell layer in mice (Cunningham et al., 1997). In human tissues, both TNFR1 and TNFR-2 receptors were found in normal vitreous, while in cultured retinal pigment epithelium (RPE) cells, ELISA showed soluble TNFR1 but not TNFR-2 (Sippy et al., 1996). Also in human tissue, retinal immunostaining for the cytokine TNF-A has been predominantly positive in glial cells, whereas the TNFR1 receptor has been mainly found in retinal ganglion cells (McKinnon, 2003; Tezel et al., 2001). Increased TNF-α production by reactivated glial cells in several retinal diseases similarly has been implicated in the death of neuronal cells. The localization of TNF-α was detected most prominently in the inner retinal layers. That agrees with the distribution pattern of the retinal glial cells, because astrocytes are primarily in the retinal ganglion cell and nerve fiber layers and cell bodies of the Müller cells are located in the inner nuclear layer. The presence of TNF-receptor-1 in the retinal ganglion cells also may indicate sensitivity to the cytotoxic effects of TNF-α. The basis for TNF functioning in the eye and the retina could possibly arise from the study of neurons. In normal brains, TNF-α may perform regulatory functions or neuromodulation, while in cerebral ischemia TNF-α may have both neurotoxic and neuroprotective functions (Fontaine et al., 2002).

4.2. TNF-α: function in systemic and eye diseases

In human disease, TNF-α may promote apoptosis/necrosis in tumor cells, immunostimulation, or defense against pathogens (McDermott, 2001; Tracey et al., 2008). Experimental data strongly support the role of the TNF-α subtype and TNFR families in the pathogenesis of systemic inflammatory, infectious, neoplastic and autoimmune diseases of lung, joints, breasts, and hematopoietic tissue (Shu et al., 1997). In addition, the central pathogenic role of TNF in medicine is supported by the clinical efficacy of TNF-α-antagonists such as infliximab in randomized controlled trials for various diseases including rheumatoid arthritis (RA) and Crohn's disease (Scott and Kingsley, 2006). Furthermore, although TNF is barely detectable in the serum of healthy humans at levels of 10 fg/ml, in patients with systemic inflammatory or neoplastic diseases, TNF serum levels increase markedly to 50 pg/ml (Edrees et al., 2005).

Compared with TNF-α, less research is available to clarify the role of TNF-β in human diseases. TNF-B is predominantly involved

in the formation of lymphoid and microvascular tissues during development, although TNF-B may act on a plethora of different cells (Ettinger et al., 1998). For instance, TNF-B may be cytolytic for many cancerous cells, while it promotes the proliferation of fibroblasts and thereby participates in wound healing. Hemorrhagic tumor necrosis induced by TNF-β may result from inhibited proliferation of endothelial cells and the activity of TNF-β as an antiangiogenic factor. Studies should be conducted to clarify the function of that cytokine for ocular tissues.

The cytokine TNF-α may participate actively in the pathogenesis of inflammatory, edematous, neovascular and neurodegenerative ocular, and extraocular diseases. Intraocular TNF-α injection in animals induces inflammation and breakdown of the blood–retina barrier that manifests as uveitis, and increased levels of TNF-α and TNF-receptors have been found in serum and ocular fluids in rats, mice, and humans with uveitis (Derevjanik et al., 2002; de Vos et al., 1994; Santos Lacomba et al., 2001). Involvement of TNF-α in uveitis has been supported further by two animal models, experimental autoimmune uveitis (EAU) and endotoxin-induced uveitis (EIU). In EAU, the inhibition of TNF-α suppresses the so-called Th1-effector mechanism, inactivates macrophages, and leads to the destruction of ocular tissues. Moreover, some regulatory function of TNF-α on the inflammatory proliferative response has been proposed during experiments with the EAU model. In EIU, mice deficient in TNF-receptors have decreased ocular inflammatory changes (Smith et al., 1998). A central role of TNF in ocular inflammation was supported further by genetic findings; disturbed TNF-α expression through TNF-α gene polymorphisms and increased numbers of leukocytes that overproduce TNF were found in patients with Behcet's disease (Ahmad et al., 2003). Based on previous data, TNF-α therapy seems to modulate peripheral blood T-cells in patients with posterior ocular inflammation and in theory could be a reasonable approach to restoring vision (Greiner et al., 2004).

In contrast to these findings, TNF inhibition in animals has failed to block neutrophil infiltration in EIU, which may be explained either by the participation of other inflammatory cytokines or the possible role of the TNF cytokine earlier in disease development (Rosenbaum and Boney, 1993). In this context, the primary sources of TNF-α in immunity and inflammatory diseases (cells of the monocyte/macrophage lineage) may secrete TNF-α in response to exogenous molecules such as lipopolysaccharide and endogenous mediators such as interleukin (IL-1) and interferon. This observation supports the theory that TNF simply may be an intermediate player in the complex network of inflammation, whereas there might be a small role for cytokines as primary or main causes of disease initiation.

Moreover, recent evidence has emerged regarding the interplay of TNF-α in the pathogenesis of experimental retinal neovascularization, proliferative vitreoretinopathy (PVR), and macular edema based on data obtained from animal models and tissues obtained during vitreoretinal surgery (Grant et al., 2004; Limb et al., 2001; Theodossiadis et al., 2007). For instance, the pro-angiogenic inflammatory cytokine TNF-α may stimulate preretinal neovascularization during retinal hypoxia in a murine model of oxygen-induced retinopathy (Gardiner et al., 2005). In addition, in cultured choroidal endothelial cells TNF-α modulates endothelial plasticity and survival by sequential inactivation of Tie2 followed by activation of Tie2 and VEGF receptors (Hangai et al., 2006). TNF also may exert proliferative and apoptotic activity in entities with proliferative membranes such as PVR associated with rhegmatogenous retinal detachment or proliferative diabetic vitreoretinopathy.

Regarding inflammation in external ocular tissues, previous studies have confirmed a similar role for TNF-α. In conjunction with other cytokines, TNF-α can mediate a wide array of immune functions in addition to up-regulation of protease expression and control of corneal destruction. Inhibition of TNF activity prolonged corneal allograft survival in animal models, providing additional therapeutic perspective (Rayner et al., 2000). In addition, infiltration of TNF-α producing T-cells may participate actively in extraocular inflammation such as thyroid-associated ophthalmopathy, vernal keratoconjunctivitis, or scleritis (Kapadia and Rubin, 2006; Durrani et al., 2005; Leonardi et al., 2003). Anti-TNF may have a limited role in the treatment of extraocular autoimmune inflammatory diseases. Besides its proinflammatory effect, TNF-α is an immunoregulator that diminishes or prevents autoimmunity. This dual physiologic role as a proinflammatory and immunoregulatory mediator might explain why anti-TNF-α therapies possibly could deteriorate the autoimmune status in some patients.

There is an unclear relationship between TNF cytokine and neuronal retinal diseases. TNF- and TNF-receptor-1 are found in glial and retinal ganglion cells, respectively. The localization of these structures may explain partly the increased sensitivity to the cytotoxic effects of TNF-α degeneration in glaucoma (Tezel et al., 2001, 2004). Experimental studies using cultures of retinal ganglion cells and glial cells provided direct evidence that elevated pressure or ischemia can initiate the cell apoptosis cascade in retinal ganglion cells, largely through TNF-α secreted by reactivated glial cells in response to these stressors. In addition, up-regulation of TNF-α and its type-1 receptor in patients with glaucomatous retina suggests that TNF-mediated cell death is involved in the neurodegeneration process of glaucoma, although no cause–effect relationship has been established (Fontaine et al., 2002; Tezel et al., 2001). Furthermore, retinal ganglion cell death in cultures can be attenuated by about 66% due to inhibition of the TNF bioactivity. Interestingly, TNF in the retina of normal eyes may protect retinal cells and optic nerve axons from apoptosis through an NF-kappaB-dependent mechanism in the physiologic state and in degenerative retinal diseases such as AMD (Tezel et al., 2004; MacEwan, 2002). This variety of data suggests that cytokines have a relevant effect on neuronal tissue and its pathologic states, although their exact role has not been clarified.

4.3. TNF-α: Infliximab

4.3.1. Infliximab: characteristics and function in systemic diseases

Infliximab (Remicade®, Centocor, Horsham, PA, USA) is a chimeric human immunoglobulin IgG1 with a mouse Fv variable fragment with high TNF-α affinity and neutralizing capacity. It is produced by a recombinant cell line in culture medium and purified with viruses resulting in a 149-kDa chimeric mAb (Calabrese, 2003). Infliximab binds to both the transmembrane and soluble form of human TNF-α with an association constant of 1010 M and a serum half-life of 10 days, but it does not neutralize the isoform TNF-β (Nestorov, 2005).

Infliximab inhibits functional TNF-α activity in a variety of in vitro bioassays using human fibroblasts, endothelial cells, neutrophils, lymphocytes, and epithelial cells (Macías et al., 2005; Scallon et al., 2002). Evidence supports the idea that the main mechanism of action of infliximab in arthritis therapy involves the induction of apoptosis of T-cells, monocytes, and macrophages. In vivo infliximab prevented the development of arthritis in a transgenic mouse model, whereas in humans the drug is a highly effective FDA-approved treatment for TNF-driven diseases such as inflammatory arthritis and Crohn's disease (Edrees et al., 2005). Intra-articular injections with infliximab can be used in mono or oligoarthritis in RA to achieve rapid and pronounced, although temporary, suppression of local joint inflammation (Conti et al., 2005). Paradoxically, a systemically administered anti-TNF agent decreases TNF-α activity but increases the TNF-α half-life. This may be explained theoretically by several mechanisms such as a reactive over-transcription of TNF cytokine, stimulation of a parallel pathway that produces members of the TNF family, or the coupling of an mAb target that may increase the permanence of the TNF

cytokine in the circulation. Collectively, although clinical data strongly suggest infliximab to be a powerful agent for controlling inflammation in human disease, TNF inhibitors are not the immunologic cure for inflammatory rheumatic diseases such as RA.

4.3.2. Infliximab: role in inflammatory ocular diseases

Consecutive studies have described the role of endogenous infliximab in the treatment of ocular inflammation. Single or multiple infusions of infliximab at concentrations of 3–10 mg/kg within a 2- to 36-month period have been efficacious in preventing ocular attacks, decreasing relapses, diminishing concomitant corticosteroid use, and controlling disease activity in patients with idiopathic uveitis or uveitis associated with juvenile arthritis, ankylosing spondylitis, Behcet's disease, sarcoidosis, or Crohn's disease (Table 2) (Abu El-Asrar et al., 2005; Benitez-del-Castillo et al., 2005; Bodaghi et al., 2005; Braun et al., 2005; Deuter et al., 2007; El-Shabrawi and Hermann, 2002; Falappone et al., 2004; Kahn et al., 2006; Lanthier et al., 2005; Markomichelakis et al., 2004; Murphy et al., 2004; Nakamura and Ohno, 2005; Niccoli et al., 2007; Ohno et al., 2004; Rajaraman et al., 2006; Richards et al., 2005; Saurenmann et al., 2006; Suhler et al., 2005; Sfikakis et al., 2004; Tugal-Tutkun et al., 2005; Wechsler et al., 2004). Reduced signs of ocular inflammation have been reported in 50–100% of patients with uveitis secondary to various causes. One prospective clinical trial reported the short-term (10-week) efficacy of infliximab in 78% of patients treated for refractory autoimmune uveitis (Suhler et al., 2005). Recent studies including one from a prospective phase 2 trial reported that patients with uveitis associated with Behcet's disease had the fastest improvement of ocular inflammatory signs and visual acuity (VA) with multiple higher-dose (5 or 10 mg/kg) infliximab infusions (Baughman et al., 2005; Evereklioglu, 2005; Giansanti et al., 2004; Lindstedt et al., 2005; Ohno et al., 2004; Sfikakis et al., 2001; Tugal-Tutkun et al., 2005). However, recent data have shown that patients with Behcet's disease may require repetitive infliximab infusions to maintain an adequate clinical response (Sfikakis et al., 2004). An ongoing clinical trial is evaluating the effect of infliximab in patients with uveitis that is refractory to other forms of systemic immunosuppressive therapy (http://www.clinicaltrials.gov/ct/show/NCT00273390?order=1). This trial should show the precise indication of infliximab for uveitis. The expected results from studies of treatment of these patients include whether infliximab may be used as a first-line agent, whether there are specific side effects when infliximab is used to treat ocular inflammation, the best time to start anti-TNF therapy in patients with uveitis, and which inflammatory ocular conditions may benefit from therapy with chimeric mAbs. Infliximab has become an important agent in the treatment of refractory uveitis, especially in severe diseases such as Behçet's disease.

Besides uveitis, some case series reported the off-label use of infliximab to treat extraocular inflammatory diseases including myositis, Sjögren's syndrome, thyroid or Wegener's orbital inflammation ophthalmopathy, optic neuritis, and scleritis in patients in whom traditional therapy with radiotherapy or anti-inflammatory medications have failed (Ashok et al., 2005; Cazabon et al., 2005; Diaz-Valle et al., 2004; Durrani et al., 2005; El-Shabrawi and Hermann, 2005; Garrity et al., 2004; Pessler et al., 2006; Rosenbaum and Smith, 2002; Rubin and Foster, 2004; Santos Lacomba et al., 2004; Wilson et al., 2004). A recent publication suggested that idiopathic intraocular inflammatory conditions such as birdshot choroiditis and Vogt-Koyanagi-Harada may be potential targets for anti-TNF therapies (Baughman et al., 2005); little evidence supports the use of infliximab for such rare diseases. However, use of anti-TNF may be considered since no other effective therapy exists.

Whereas infliximab has been used only systemically, in the future anti-TNF mAbs may be used intraocularly to manage severe intraocular inflammatory and angiogenic diseases. Intravitreal injections of infliximab at concentrations below 1.25 mg were safe for retinal tissue in preliminary animal studies (Kazi et al., 2006), while another study reported its safety up to 1.7 mg (Giansanti et al., 2008). Future investigations of intravitreal infliximab include tests of intravitreal efficacy in animal models of uveitis, retinal edema or neovascularization; investigation of retinal safety and penetration in a primate model (we just initiated experiments in the South American marmoset primate for this purpose, preliminary data showed functional damage with the higher dose of 400 μg detected by electroretinography); examination of the distribution and absorption of periocular (peribulbar or sub-Tenon) injections of infliximab; and execution of phase 1 trials in humans to examine the safety profile, our study group is performing a phase 1 trial (Infliximab for Diabetes with Edema of the Macula, named READ Study), with two intravitreal injections of 1 mg of infliximab in patients ($n = 10$) with diabetic macular edema (DME) who will be followed for 6 months; results are expected within 24 months.

4.4. TNF-α: etanercept, adalimumab, and certolizumab pegol

Etanercept (Enbrel®, Amgen, Thousand Oaks, CA, USA) is an engineered recombinant dimeric protein generated by the fusion of ligand-binding portion of human TNFR-2 linked to the Fc portion of human IgG1 (Zhou, 2005). Etanercept has a short half-life (about 4 days), and it may not induce antibody neutralization and cause lysis with or without complement. Etanercept is not a mAb agent, although it has been termed an antibody-like drug. It can be used to treat the same inflammatory ocular diseases treated with other anti-TNF mAbs. However, other anti-TNF mAbs also may be considered before choosing this drug as the first treatment option. The soluble drug acts competitively by inhibiting the binding of TNF-α and TNF-β to their cell-surface receptors. Subcutaneous injection of 25 mg of etanercept yielded clinical stabilization mainly for RA and systemic juvenile idiopathic arthritis and received FDA approval in June 2003 (Nanda and Bathon, 2004). In rats with endotoxin-induced uveitis, subcutaneous etanercept promoted reduction in aqueous TNF levels and control of intraocular inflammation (Avunduk et al., 2004). The clinical benefits in humans have been observed with subcutaneous injections of etanercept twice weekly in children with juvenile idiopathic arthritis and associated uveitis (Reiff et al., 2001; Smith et al., 2001). Moreover, combining etanercept with methotrexate resulted in better inflammatory control than methotrexate or etanercept alone in the maintenance of uveitis remission (Smith et al., 2001; Foster et al., 2003). However, treatment with etanercept only in children with a high-risk oligoarticular and seronegative polyarticular arthritis did not change the frequency of episodes of recurrent uveitis (Schmeling and Horneff, 2005). Interestingly, there have been more reports about etanercept for treatment of uveitis associated with juvenile idiopathic arthritis than infliximab (Imrie and Dick, 2007). Past clinical experience has suggested that etanercept could be a good option for treating children and young adults compared with infliximab or adalimumab; however, further studies are necessary to confirm this issue. Despite a good response when used to treat inflammatory uveitis, paradoxically, systemic etanercept could worsen the ocular inflammation in some patients. It is important to follow inflammatory signs before and after etanercept administration and be aware of other important complications, such as tuberculous uveitis. The most common side effect of etanercept is a reaction at the injection site, which can affect 37% of patients. Signs and symptoms include hyperemia, itching, and swelling. Although first reports have shown that this drug is relatively safe, more studies are necessary.

The pharmacokinetics and safety of intravitreal etanercept delivery at a dose of 100 μg have been investigated in rabbits in one study. Clinical examination, electroretinographic findings and

Table 2
Overview of the clinical use of infliximab for treatment of uveitis.

Authors, year	Design of study	Dose (mg/kg)	Number of patients	Eye segment involved	Types of uveitis	Previous immunosupression or corticoterapy (%)	Adjuvant immunosupression	Total number of applications	Final success – reduction in clinical inflammation (%)	Follow-up – months	Complications
El-Shabrawi and Hermann, 2002	Prospective non-comparative controlled	10	7	Anterior	HLA-B27 related	Yes (85)	No	1	100	17	No
Joseph et al., 2003	Prospective non-comparative controlled	5	5	Posterior	BD; idiopathic	Yes	Yes	3	80	6	Tuberculosis (1)
Sfikakis et al., 2004	Prospective non-comparataive controlled	5	25	Posterior	BD	Yes (100)	Yes	1	100	1 and 7	No
Murphy et al., 2004	Retrospective non-comparative	3 or 5	7	Anterior/posterior	Intemediate; RV&P; juvenil anterior	Yes	Yes	2–19	85	4–22	Hypersensitivity
Ohno et al., 2004	Prospective non-comparative controlled	5 or 10	13	Posterior	BD	Yes	No	4	77	4	Tuberculosis (1)
Markomichelakis et al., 2004	Prospective non-comparataive controlled	5	14	Posterior	Idiopathic; BD; vascular pseudotumor; HLA-B27-related	Yes	No	1–2	100	6	No
Wechsler et al., 2004	Retrospective non-comparative	5	4	Anterior/posterior	BD	Yes	Yes	4–15	100	22	No
Falappone et al., 2004	Retrospective non-comparative	5	5	Anterior/posterior	Spondyloarthropathy; BD	Yes (100)	Yes	8	100	6–19	No
Benitez-del-Castillo et al., 2005	Prospective non-comparative uncontrolled	5	7	Posterior	BD; sarcoidosis; IMC	Yes (100)	Yes	3	100	36	No
Lindstedt et al., 2005	Prospective non-comparative uncontrolled	3	13	Anterior/posterior	BD; idiopathic; sarcoidosis; BCR	Yes (100)	No	1–12	100	24	Atopic dermatitis (1); rash (1)
Nakamura and Ohno, 2005	Prospective non-comparative controlled	5 or 10	13	Posterior	BD	Yes (100)	No	4	100	3–8	Diarrhea, cold, malaise, nausea, pyrexia, headache, increased blood pressure, vomiting, arthralgia, constipation
Richards et al., 2005	Retrospective non-comparative	5 and 10	6	Anterior	Juvenil; idiopathic	Yes (100)	Yes	3–21	100	3–26	Pytiriasis, pruritus, staphylococus foliculitis
Lanthier et al., 2005	Retrospective non-comparative	5	4	Posterior	BD	Yes (100)	Yes	3–6	50	2–29	Cutaneous herpes
Tugal-Tutkun et al., 2005	Prospective non-comparative controlled	5	13	Posterior	BD	Yes	Yes	4	31	6–12	Respiratory tract infection, headache, hypertension, infusion reaction, rash, dermatis, back pain, inguinal hernia
Suhler et al., 2005	Prospective non-comparative controlled	3, 5, or 10	23	Intermediate/panuveitis/posterior	Idiopathic; sarcoidosis; BSCR; BD; PP; CD; MFC	Yes (100)	Yes	3	50	2–12	Pulmonary embolus, CHF, lupus-like reaction (2), vitreous hemorrhage (2)
Bodaghi et al., 2005	Retrospective non-comparative	5 or 10	21	Anterior/posterior	BD; ankylosing spondylitis; psoriatic; idiopathic	Yes (100)	No	3 or more	100	17	No
Baughman et al., 2005	Retrospective non-comparative	[a]	14	Anterior/posterior	Various	Yes	ND	[a]	93	24	[a]

(*continued on next page*)

Table 2 (*continued*)

Authors, year	Design of study	Dose (mg/kg)	Number of patients	Eye segment involved	Types of uveitis	Previous immunosupression or corticoterapy (%)	Adjuvant immunosupression	Total number of applications	Final success – reduction in clinical inflammation (%)	Follow-up – months	Complications
Saurenmann et al., 2006	Retrospective non-comparative	3–10	24	Anterior/posterior	Childhood	Yes (100)	Yes	[a]	92	1–19	Cataract, glaucoma with etanercept; forearm infection cellulitis
Abu El-Asrar et al. 2006	Prospective non-comparative controlled	5	6	Anterior/posterior	BD	Yes (100)	Yes	4–23	50	36	ANA antibodies (33%)
Rajaraman et al., 2006	Retrospective non-comparative	5 or 10	6	Anterior/posterior	Idiopathic, RA-associated	Yes (100)	Yes	[a]	100	7–17	Vitreous hemorrhage (1); upper respiratory reaction (1)
Kahn et al., 2006	Retrospective non-comparative	10 or 20	17	Anterior/posterior	Childhood	Yes (100)	Yes	2–7	100	24	No
Niccoli et al., 2007	Prospective non-comparative controlled	5	12	Posterior	BD	Yes (100)	Yes	9	78	24	Headache, hypotension (25%); respiratory tract infection (33%)

RA = Rheumatoid arthritis; BSCR = Birdshot chorioretinopathy; MFC = multifocal choroiditis; RV&P = Retinal vasculitis and panuveitis; CD = Crohn disease; PP = pars planitis; IMC = idiopathic multifocal choroiditis.
[a] Not available.

histologic examination showed no toxicity, and retinal concentrations were at a maximum level at 4 weeks and still detectable after 8 weeks (Fauser et al., 2004). Another study of the intraocular safety of different doses of etanercept showed that the anti-TNF mAb in doses up to 2.5 mg caused no retinal damage (Kivilcim et al., 2007). Information from experimental studies on the safety of intravitreal etanercept may provide more accurate information regarding its use. Issues that should be investigated include the retinal safety in humans, the efficacy in inhibiting inflammation compared with infliximab, and the minimal concentration to be injected intravitreally or subcutaneously to inhibit intraocular inflammation.

Adalimumab (Humira®, Abbott, Chicago, IL, USA) is a fully human mAb recombinant produced by phage-display composed of two kappa light chains and two IgG1 heavy chains with molecular weight of 148 kDa (Weisman et al., 2003). Adalimumab binds specifically with high affinity to TNF-α and neutralizes its biologic function by blocking binding to p55 and p75 cell-surface receptors. Treatment of RA with subcutaneous administration of adalimumab at 40 mg every 2 weeks is FDA-approved. The drug also can be used to treat ankylosing spondylitis, Crohn's disease, and psoriasis (Furst et al., 2003). Regarding ocular diseases, several recent case series reported the clinical benefits of adalimumab to treat uveitis related to Behcet's disease and its childhood type of Behcet's disease (Biester et al., 2007; Vazquez-Cobian et al., 2006; Foeldvari et al., 2007). One clinical trial, Adalimumab in Uveitis Refractory to Conventional Therapy (ADUR Trial, available from www.clinicaltrials.gov), is recruiting patients to evaluate the efficacy of adalimumab in patients with different forms of refractory uveitis. More consistent results are necessary to define its role and set guidelines for treating intraocular inflammation (Biester et al., 2007; Vazquez-Cobian et al., 2006; Foeldvari et al., 2007), The minimum adalimumab dose necessary to inhibit intraocular inflammation may not be the same as that used to treat systemic inflammation. Biweekly administration of 40 mg is the usual dose indicated for arthritis. For uveitis, doses as low as 20 mg, 10 mg, or less every 2 weeks may be sufficient to control inflammation.

Adalimumab has a great advantage over infliximab because it is a fully humanized antibody. For intravitreal use, it is important that adalimumab promotes inflammatory reactions rarely; surgeons have observed similar rare immunogenic responses with bevacizumab and ranibizumab. The ocular pharmacokinetics and retinal safety of adalimumab investigated recently in a rabbit model (Manzano et al., 2008) showed no signs of toxicity in the groups injected with 0.25 mg or 0.50 mg adalimumab. However, inflammation, retinal necrosis and reduction in a-waves and b-waves were present in two out of three eyes that received 1.0 mg of adalimumab. Compared to human eyes, assuming a vitreous volume three times larger (4.5 ml) than that of rabbits, possibly up to 0.75 mg, 1.5 mg, and 3.0 mg of adalimumab can be injected. One drawback of this study was that adalimumab is a human IgG, and therefore, we hypothesized that inflammatory reactions would be less likely in human than rabbit eyes. However, there have been no such reactions in rabbit eyes injected with another humanized mAb (bevacizumab). To address the issue of retinal safety and penetration of anti-TNF mAbs, we are conducting a study with intravitreal injections of adalimumab and infliximab in the New World primate, Common Marmoset (*Callithrix (Callithrix) jacchus*). This study also aims to examine the penetration of anti-TNF mAb and may provide important information regarding intraocular administration of adalimumab. The importance of this study lies in the fact that intravitreal adalimumab may offer lower costs than its systemic administration, as it also promises to be a good effective alternative to manage intraocular inflammation. More experimental studies should evaluate its use for neovascular diseases and macular edema.

Certolizumab pegol (Cimzia®, Nektar/UCBPharma, San Carlos, CA, USA, and Brussels, Belgium) is a PEGylated Fab fragment of a high-affinity humanized anti-TNF mAb, which has shown positive results in phase 3 trials of Crohn's disease and RA. The mAb is injected subcutaneously (400 mg) every 2 weeks and every 4 weeks as maintenance therapy (Kaushik and Moots, 2005). To date, the efficacy has not been directly compared to other anti-TNF-α agents for the management of any systemic disease. By November 2008, there were no data available in the literature on the use of certolizumab pegol to treat ocular diseases. Thus, no formal recommendations regarding the use of this drug can be provided.

4.5. Novel and controversial issues of anti-TNF mAbs therapy

The distinct pharmacology and efficacy profiles of TNF inhibitors raise numerous controversies regarding their use in ophthalmology (Table 3). Systemic infliximab possesses greater bioavailability and higher drug concentrations than the soluble etanercept. However, the disadvantages of infliximab therapy include the short duration of effect (less than 6 weeks), the necessity for repeated treatments, and high cost (Furst et al., 2006). A recent retrospective analysis reported overall enhanced effectiveness of systemic infliximab over etanercept in controlling ocular inflammation (Braun et al., 2005; Galor et al., 2006). However, most studies that evaluated infliximab and etanercept had a small sample size, retrospective data collection, and heterogeneity of underlying diseases and patients.

The efficacy of anti-TNF agents varies according to the type of systemic arthritis; heterogeneous effects also can be observed in the treatment of uveitis. Regarding specific diseases, infliximab may have a stronger effect in patients with uveitis associated with Behcet's disease, Crohn's disease, sarcoidosis, and spondyloarthropathy. The advantages of infliximab over etanercept to treat uveitis include better ocular penetration or a greater effect on the immune system due to apoptosis promotion in TNF-α expressing cells. Moreover, for young patients with uveitis, the novel adalimumab may provide better clinical control than infliximab. Recently, Foeldvari et al. reported results from their multicenter study on the use of anti-TNF agents to treat juvenile idiopathic RA. In that study, infliximab was significantly ($p = 0.004$) more efficacious for the treatment of juvenile idiopathic arthritis-associated uveitis than etanercept (Foeldvari et al., 2007).

The differences in mAb efficacy may be related to disease pathophysiology (e.g., more important role of other cytokines such as lymphotoxin IL-1), host factors (e.g., ethnicity, concomitant diseases), and drug characteristics (e.g., dose, pharmacokinetics, immunogenicity, ability to block lymphotoxin or fix complement, or the propensity to induce apoptosis) (Calabrese, 2003). Biologic differences in gene expression among several vascular and non-vascular cells in different tissues may contribute to the differences in the responses to mAbs. In the eye and other organs, molecular and cellular elements interact to promote tissue repair in a disease state. Inhibiting one specific component of the process could result in disease control but also could lead to different biochemical pathways, inducing an even more severe pathological state. In addition, the action of different cellular and molecular mediators could vary in every patient at different disease stages.

Table 3
Pharmacologic and clinical properties of three TNF inhibitors.

	Infliximab	Etanercept	Adalimumab
Binds to free TNF-A	Yes	Yes	Yes
Binds to TNF-A on cell membranes	Yes	No	Yes
Binds to TNF-B	No	Yes	No
Half-life (days)	8	4	14
Route of administration	Endovenous	Subcutaneous	Subcutaneous
Volume of distribution (l)	3	10.4	5
C_{max} (μg/ml)	118	1.1	4.7
Time to C_{max} (h)	NA	69	131
Application frequency	Every 8 weeks	Twice a week	Every 2 weeks

Although anti-TNF agents reduce the signs of ocular inflammation, there has been conflicting evidence whether neutralizing TNF activity during the effector phase or the affector stage of uveitis suppresses histologic damage and improves VA. Further studies are needed to determine the first-line TNF-blocker agent for each type of intraocular inflammatory disease and whether adjuvant therapy with immunosuppressants such as cyclosporine and methotrexate is necessary. Moreover, the optimal dose, administration route, and dose regimens for anti-TNF mAb administration also should be investigated (Smith et al., 2001).

Interestingly, anti-TNF mAb treatment may be used as adjuvant therapy in some mainly VEGF-mediated intraocular neovascular diseases including macular edema and choroidal neovascularization (CNV) (Sfikakis et al., 2005). In patients with macular edema secondary to uveitis and DR, systemic infliximab reduced macular thickness and promoted vision recovery as reported in some case series. In an EIU-model, TNF inhibition decreased breakdown of the blood–retinal barrier (Sfikakis et al., 2005; Markomichelakis et al., 2005). The reduction of the blood–retinal barrier breakdown after infliximab therapy may be explained either by a leukocyte-mediated TNF-α effect on vascular injury through the Fas/FasL pathways or by an indirect effect on other vascular regulatory cytokines. However, in a small case series, seven patients with refractory DME received two intravitreal injections of etanercept 2.5 mg without any clinical improvement (Tsilimbaris et al., 2007). Anti-TNF mAbs probably could be useful as adjuvant treatment in macular edema, especially in chronic and refractory cases.

Regarding ocular neovascularization, one patient with Behcet's disease with uveitis and retinal neovascularization treated with systemic infliximab had regression of new vessels after 8 months. A series of patients receiving 5 mg/kg of infliximab infusions for inflammatory arthritis had remarkable regression of CNV due to AMD (Pessler et al., 2006; Vazquez-Cobian et al., 2006). The preventive and therapeutic effects of infliximab and etanercept have been studied in a rat model of laser-induced CNV as reported previously by other reports and by our research group (Olson et al., 2007). In the study by Olson et al., both anti-TNF agents given prophylactically decreased the size and leakage of CNV lesions in these animal models, although in one study only etanercept induced reduction of CNV (Shi et al., 2006). We performed intravitreal injection of escalating doses of infliximab from 10 to 320 μg in rats after laser-induced CNV. At lower doses, infliximab promoted significant reduction of neovascular complex. However, at higher doses, it induced no effect compared to the control group (Fig. 3). These results suggested that either the pro-angiogenic effect of anti-TNF mAb may occur only at lower doses or that in a higher dose some antiangiogenic indirect effect may be seen. Clinical studies have shown a marked elevation in vitreous levels of TNF-α in patients with PDR (Limb et al., 1999; Doganay et al., 2002). Experimental studies in a rat DR model showed that anti-TNF agents may reduce leukocyte adhesion, blood–retina barrier breakdown, and endothelial injury. The association between TNF-α and pathologic intraocular neovascularization may be explained by direct transmembrane-TNF stimulation of blood vessel growth, or TNF-α-induced expression of isoform VEGF-C, which may protect retinal endothelial cells from apoptosis (Zhao et al., 2007).

Anti-TNF agents should be examined in pre-clinical and human studies of diseases that have no consensus about the best treatment option, such as refractory PDR or PVR. Limb et al. (2001) reported that TNF-receptors and TNF-α are elevated in cases with retinal

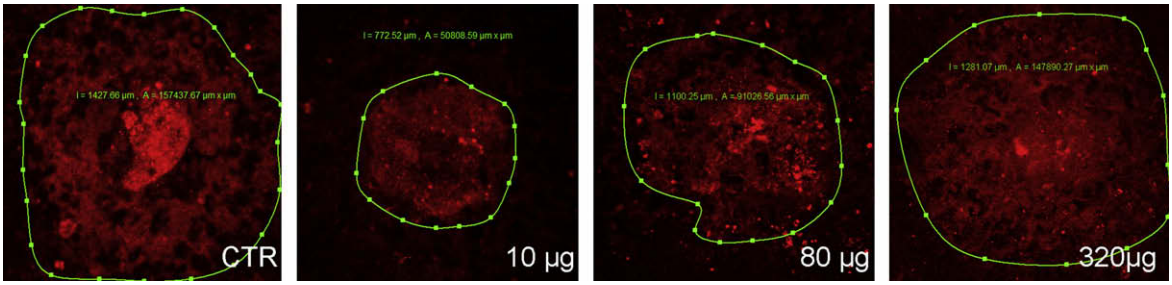

Fig. 3. Confocal analysis of flat-mount retinal tissue using immunofluorescence with anti-von Willebrand Factor (vWF) in CNV laser-induced lesions before and after intravitreous infliximab treatment. A stronger antiangiogenic effect of anti-TNF agent has been achieved with application of the lower dose 10 µg. VWF is a specific marker for endothelial cells. The line in green shows perimeter and area of the lesions.

detachment, tractional retinal detachment, and especially in PVR. PVR is an important cause of retinal redetachment and no effective treatment exists.

In summary, anti-TNF drugs clearly have a role in controlling intraocular inflammation, although future studies are needed to elucidate the precise role of anti-TNF mAbs in neovascular, proliferative, and edematous lesions, which may lead to novel therapies for ophthalmic clinical practice.

4.6. Safety issues regarding anti-TNF therapy in ophthalmology

4.6.1. Systemic safety of anti-TNF mAbs

Important information about the side effects of TNF inhibitors emerged from randomized clinical trials in patients with inflammatory joint diseases. During and immediately after intravenous infusions of infliximab, mild allergic reactions can develop at the infusion or injection site in about 5% of cases. Post-injection adverse effects of various anti-TNF agents include malignancies, lupus-like reaction with autoantibody formation, demyelinating neurologic diseases such as exacerbations of multiple sclerosis, pulmonary embolus, worsening of congestive heart failure, early and delayed autoimmunity hypersensitivity reactions, and cell-mediated infections including active tuberculosis (Suhler et al., 2005). Regarding infection, case reports of pulmonary listeriosis, pulmonary aspergillosis, *Pneumocystis carinii* pneumonia, and reactivated histoplasmosis have been published (Tai et al., 2002). Tuberculosis has been the most common serious infection associated with TNF antagonists, as its incidence is associated with age, concomitant immunosuppressive regimens, low socioeconomic status, and geography. It also has been observed that infliximab has a relatively higher risk of these side effects than etanercept.

Another major concern involving anti-TNF mAbs is the development of an immunogenic response or the so-called human antichimeric antibodies (HACA) response. These agent-related hypersensitivity events may be type I, II, or III reaction and are explained by the production of IgG antibodies against the murine proteins of infliximab (Nestorov, 2005). In patients treated with TNF inhibitors, this phenomenon is not dose dependent. These commonly isolated serum autoantibodies are antinuclear, anti-double-stranded DNA, and anticardiolipin. However, such reactions could be prevented by concomitant use of immunosuppressants such as corticosteroids. The production of HACA against biologic agents depends on several factors beyond the antigenic aspect of the protein, including the route of administration (e.g., subcutaneous vs. intravenous), treatment protocols (e.g., continuous vs. intermittent), and the concomitant use of immunosuppressive medications. Hwang and Foote (2005) grouped immunogenicity against murine, chimeric, and humanized mAbs in humans into three categories: negligible (less than 2%), tolerable (less than 15%), and marked (more than 15%). A marked HACA response was found in 84% of patients among the 44 murine MAbs, 40% among 15 chimeric antibodies, 9% of 22 humanized mAbs; the data clearly showed that chimerization and humanization significantly decreased the immunogenicity of therapeutic mAbs.

When evaluating the immunogenicity of humanized, chimeric, and human antibodies, it may be noticed the absence of a rational quantitative scale for immunogenicity, inability to compare relative immunogenicity between molecules, incomplete clinical data on immunogenicity, and failure to relate immunogenicity to molecular structure/properties. Emerging technologies and trends may help to solve this situation. Immunogenicity appears to be associated with an increased incidence of infusion reactions and shorter duration of clinical response. Potential methods of reducing antibody formation and optimizing treatment include the concomitant administration of immunosuppressive agents, such as methotrexate, and increased dosing frequency. The dual physiologic roles of TNF-α, as proinflammatory and immunoregulatory agent, might explain why anti-TNF-α therapies present a complex picture: the therapies are effective for most autoimmune patients with RA with end-organ destruction due to inflammation, but they worsen or induce autoimmunity for most of these patients. Evidence from clinical trials indicates that anti-TNF-α therapies can, under certain circumstances, promote rather than quell certain forms of autoimmunity. The evidence is strongest for multiple sclerosis, with studies showing that anti-TNF-α therapies exacerbate the disease course. One important downside of anti-TNF mAbs is that demyelinating diseases may worsen with these drugs and this risk must be considered for patients with intermediate uveitis.

4.6.2. Ocular safety of anti-TNF

To date, large clinical trials have poorly addressed ocular complications after systemic use of anti-TNF mAbs. The neuro-ophthalmic toxic effects of systemic infliximab, adalimumab, and etanercept have manifested as either anterior optic nerve neuropathy or oculomotor nerve palsy. In addition, over 15 cases of optic neuritis have been reported (Suhler et al., 2005; Chung et al., 2006; Theodossiadis et al., 2007). Ocular side effects such as cataract, infection, or increased intraocular pressure (IOP) have not been observed, although one study has suggested the occurrence of mild vitreous hemorrhage in two patients (Suhler et al., 2005; Rajaraman et al., 2006).

5. Use of anti-VEGF mAbs in ocular diseases

5.1. VEGF: structure and biology in human health

VEGF is a family of proteins that includes placenta growth factor (PlGF), VEGF-A, VEGF-B, VEGF-C, VEGF-D and the viral VEGF

homologue VEGF-E (Ferrara et al., 2001, 2003). The well-studied VEGF-A is a dimeric 36–46 kDa glycosylated protein comprising nine different isoforms in humans generated from alternative mRNA splicing. A total of four well-studied isoforms include $VEGF_{121}$, $VEGF_{165}$, $VEGF_{189}$, and $VEGF_{206}$. VEGF-A isoforms induce proliferation, migration and tube formation of vascular endothelial cells through a variety of pathways with final activation of vascular protein kinases through three VEGF receptors: VEGFR-1 (Flt-1), VEGFR-2 (KDR), and VEGFR-3 (Flt-4) (Neufeld et al., 1999). In addition, VEGF-A promotes enhanced vascular permeability through the formation of specialized regions in the plasma membrane of endothelial cells that are highly permeable to macromolecules called vesicular–vacuolar organelles and fenestra (Mathews et al., 1997). VEGF promotes proliferation of vascular endothelial cells derived from arteries, veins, and lymphatic vessels through a variety of pathways by binding to VEGFR2, causing VEGF-receptor dimerization, tyrosine phosphorylation, and signal transduction for activation of mitogen-activated protein kinase (Thakker et al., 1999).

VEGF cytokines may participate in the regulation of physiologic activities during embryonic development and in healthy human adult tissues. The absence of VEGF during embryogenesis may be lethal and promote deficient retinal vascular development (Byrne et al., 2005). Although endothelial cells are the primary targets of VEGF, several studies have reported mitogenic effects on non-endothelial cell types such as RPE cells, pancreatic duct cells, and Schwann cells. In adult normal tissues, signaling of VEGF isotypes influences wound healing and participates in the female reproductive cycle (Ferrara et al., 2003).

The role for members of the VEGF family other than VEGF-A under physiologic conditions remains mostly unclear. VEGF-B is predominantly expressed in myocardium and skeletal muscle tissue; VEGF-B knockouts have reduced heart size and recover poorly from experimental myocardial ischemia (Olofsson et al., 1996; Bellomo et al., 2000). The selective VEGF-C expression profile and binding to VEGFR-3 suggest direct participation in the development of the lymphatic system during embryogenesis and its maintenance in adulthood (Eriksson and Alitalo, 1999; Lymboussaki et al., 1998; Kukk et al., 1996; Joukov et al., 1996; Kaipainen et al., 1995).

Continuous expression of VEGF and its receptors VEGFR1 and VEGFR2 in normal ocular structures has been demonstrated widely within vascularized ocular tissues including the conjunctiva, iris, retina, and choroid, suggesting that VEGF is also a survival factor for ocular vascular tissues (Kim et al., 1999; Witmer et al., 2003). However, little or no VEGF has been detected in non-pathologic avascular cornea. However, its expression increased in epithelial cells of inflamed corneas and on vascular endothelial cells. Likewise, VEGF concentrations were significantly higher in vascularized corneas compared with normal control corneas (Philipp et al., 2000).

In the normal retina, the participation of the VEGF family has been suggested by the presence of three VEGF receptors within the vascular, epithelial, and neuronal retinal elements, while various retinal cells including pericytes, ganglion cells, endothelial cells, Müller cells, and RPE cells may produce VEGF in non-pathologic states (Jin et al., 2000). In adults, VEGF prevents apoptosis of neuroretinal and vascular cells in a dose-dependent manner. VEGF is a survival factor for endothelial cells by maintaining their integrity via anti-apoptotic signaling (Thakker et al., 1999; Gerber et al., 1998). VEGF-A stimulated axonal outgrowth via VEGFR2 and has a direct neuroprotective effect on neurons after ischemic injury via VEGFR2, as proposed by Sondell et al. (2000). A recent investigation showed the role of VEGF-A in retinal neuroprotection in animals, but few animal and human clinical studies have shown little or no side effects in the neuroretina after chronic VEGF inhibition in the retina (Nishijima et al., 2007).

Interestingly, RPE cells secrete VEGF-A toward their basal choriocapillaris side, and all three VEGF receptors are expressed on the choriocapillaris endothelium facing the RPE cells. One study postulated that the most relevant receptor in the normal mammalian retinal vessels is VEGFR1. This receptor may act as a regulator of endothelial cell survival and a decoy scavenger. The staining pattern of this VEGFR1 receptor gave the strong impression of being colocalized in pericytes in vitro and in vivo (Takagi et al., 1996; Witmer et al., 2002). Moreover, VEGF-A secretion at the basolateral side increases markedly during hypoxia. The paracrine signaling interaction between the RPE and choriocapillaris through VEGF may maintain the integrity of the fenestrated permeable choriocapillaris; indeed, loss or destruction of the RPE cells in animal or human eyes causes atrophy of the choriocapillaris (Blaauwgeers et al., 1999).

In summary, understanding the role of VEGF subtypes and receptors within the normal vascular and non-vascular retinal cells is important in this realm of anti-VEGF pharmacologic therapies to establish the dosing limits and administration frequencies of therapeutic VEGF inhibitors.

5.2. *VEGF: function in systemic and ocular diseases*

VEGF-A plays a predominant role in the development of pathological angiogenesis in neoplastic, inflammatory, and vascular diseases in the eye and other organs. VEGF-A overexpression has been found in numerous clinical and experimental tumors such as carcinoma of the lungs, breast, bladder, kidney, and glioblastoma multiform; collateral cardiac vascularization after ischemia; and inflammatory tissues such as synovial fluid in RA and psoriasis lesions (Homsi and Daud, 2007). Regarding other VEGF family members, VEGF-C and VEGF-D have been extensively investigated for their role in angiogenesis and lymphangiogenesis in cancer. PlGF induces increased vascular permeability, proliferation, chemotaxis, and angiogenesis and may act synergistically with VEGF-A in angiogenesis in various pathological disorders (Luttun et al., 2002).

In the eye, VEGF-mediated angiogenesis remains an important mechanism in the pathogenesis of posterior segment intraocular diseases including DR, retinal vein occlusions (RVO), CNV, and retinopathy of prematurity (ROP) (Witmer et al., 2003). The population of VEGF-producing retinal cells in each diseased eye is likely to represent ischemic resident cells in the retina. An important driving force of hypoxia-induced VEGF-A expression in ischemic retinal disease may be the high oxygen consumption by rod photoreceptors. In vitro, RPE cells respond chemotactically to VEGF, express VEGF receptors, and produce VEGF, suggesting that VEGF may be an autocrine growth factor for RPE. Intraocular VEGF-A levels correlate with blood vessel formation in patients with DR, RVO, exudative AMD, and other retinal disorders (Campochiaro, 2007a, c). Moreover, increased VEGF-A expression in the aqueous humor, vitreous, retinal cells, and epiretinal membranes has been described in experimental models and samples of retinal tissues with proliferative and neovascular diseases (Aiello et al., 1994, 1995; Campochiaro, 2007c). These observations have appeal for modulating the retinal angiogenic response with antiangiogenic agents, for instance, VEGF inhibitors, and provide the basis for the use of anti-VEGF mAbs in diseases with proliferating fibroglial cells such as proliferative diabetic vitreoretinopathy and PVR secondary to rhegmatogenous retinal detachment. However, early studies by Aiello et al. (1995) have shown that VEGF is not the only mediator of retinal pathologic neovascularization and may be responsible for only 50% of angiogenic stimuli.

VEGF also has been related to corneal angiogenesis in herpes simplex infection as well as conjunctival, choroidal, and orbital

angiogenesis associated with and vascular/melanocytic tumors. In corneal diseases, VEGF overexpression may represent the wound healing process of end-stage diseases. Regarding ocular tumors, VEGF may facilitate formation of the neovascular net responsible for establishment of the cancerous mass, but there has been no evidence to date that inhibiting VEGF may decrease neoplastic growth or induce melanoma regression. In summary, the amount of cytokine necessary for the initiation and the pathophysiology of those conditions is still unknown (Boyd et al., 2002; Zheng et al., 2001).

VEGF is also important in the activation of vascular leakage pathways common to many diseases, such as DR, exudative macular degeneration, retinal vascular occlusions, and uveitis (Caldwell et al., 2003). VEGF-induced vascular leakage is mediated by cytoplasmic protein kinase members of the Src proto-oncogene family in the brain, heart, and other tissues. Such vascular permeability is likely related to loss of integrity in adherens junctions, which regulate cell–cell adhesion (Eliceiri et al., 1999)

5.3. Anti-VEGF: bevacizumab

5.3.1. Bevacizumab: characteristics and function in systemic diseases

Research from the 1980s and 1990s has shown that VEGF inhibition using mAb against VEGF markedly suppressed tumor growth in vivo, thereby precipitating the development of bevacizumab. Bevacizumab is a humanized mAb IgG1 against VEGF that selectively inhibits all isoforms of VEGF-A and comprised of amino acid sequences of about 93% human immunoglobulin G and 7% murine antibody. In animal models such as mice, bevacizumab inhibits the growth of human tumor cell lines with maximal inhibition at doses of 1–2 mg/kg twice weekly. Bevacizumab has been approved for first-line treatment of metastatic colorectal cancer at 5 mg/kg infusion twice monthly in combination with 5-fluorouracil, an intravenous chemotherapeutic agent; phase III clinical trials have evaluated bevacizumab in breast, lung, and kidney cancers (Hampton, 2005). No evidence of an antibody immunogenic response to bevacizumab has been found in any clinical trials, confirming the success of the humanization technique. Of clinical relevance to ophthalmology, bevacizumab cancer therapy rarely leads to tumor regression.

5.3.2. Bevacizumab: role in angiogenic and edematous ocular diseases

For ocular angiogenesis, various consecutive experimental studies have reported that bevacizumab may inhibit and prevent proliferation of cultured endothelial cells and corneal neovascularization when used topically or subconjunctivally and retinal or CNV after intravitreal injection (Bock et al., 2007; Hurmeric et al., 2008; Kaiser, 2006; Kaiserman et al., 2008; Kim et al., 2008; Manzano et al., 2007). Some of our preliminary investigations have shown that topical bevacizumab may prevent formation of new vessels in rabbit cornea. However, once neovascularization is established, mAb had little or no effect (unpublished data). We hypothesized that once neovascularization was established, the connective tissue provided mechanical support for the angiogenesis network, and endothelial cells in mature new vessels did not require VEGF as a survival factor as did immature new vessels. The large body of evidence from laboratory experiments supports the role of bevacizumab in ocular therapeutics. However, some unanswered questions remain, such as the minimum dose of bevacizumab needed to inhibit ocular neovascularization, the timing of disease for drug injection, and the pharmacokinetics of bevacizumab in the retina when intravitreally injected.

In clinical therapeutics in ophthalmology, bevacizumab has been a promising therapeutic option for targeting VEGF-related ocular neovascularization seen in various diseases such as CNV secondary to AMD, high myopia and angioid streaks, RVO, corneal neovascularization, DR, neovascular glaucoma, and ROP (Campochiaro, 2006) (Fig. 4). Whereas DR severity depends on the long-term elevated serum glucose levels, local ocular treatment relies on laser photocoagulation for patients with clinically relevant macular edema or high-risk PDR. Intraocular injection of bevacizumab in patients with PDR may reduce fibrovascular proliferation when injected before vitrectomy, thereby facilitating surgery and decreasing the risk of intraoperative hemorrhage (Fig. 3) (Friedlander and Welch, 2006). Moreover, intravitreal injection of bevacizumab in PDR may accelerate resorption of diabetic vitreous hemorrhage (Ruiz-Moreno et al., 2007; Jonas et al., 2007). However, clinical trials must confirm this hypothesis. In addition, the anti-VEGF mAb may be used intraoperatively during vitrectomy as adjuvant therapy (Arevalo et al., in press). In cases of severe PDR, early preoperative intravitreal injection of bevacizumab may lead to acute retinal detachment (Arevalo et al., 2008).

Anti-VEGF mAb may modulate vascular leakage in DME and promote clinical improvement of vision (DRCRN-Scott et al., 2007). Some studies have reported improved best-corrected VA and decreased central macular thickness. However, the results have not been sustained over 12 weeks, which may reflect the duration of the drug effect (Haritoglou et al., 2006). Another possible explanation is the reactive overexpression of VEGF receptors due to single or multiple bevacizumab injections that produce a rebound effect and worsening of the macular edema (Matsumoto et al., 2007). This phenomenon may explain why patients with diabetes need repeated doses of intravitreal bevacizumab. However, less improvement of ocular changes might be expected after a certain threshold. Further studies are needed to develop guidelines for the application of intravitreal bevacizumab, but some important conclusions may be drawn: better functional outcome is expected with bevacizumab if used during early-stage DME; better results are seen in patients without ischemia; and multiple injections are necessary. Furthermore, intravitreal bevacizumab should be compared to laser photocoagulation and intravitreal triamcinolone to investigate its efficacy and safety or whether a combination of these strategies is better.

Therapeutic options for CRVO or branch retinal vein occlusion (BRVO) include laser photocoagulation, vitrectomy, neurotomy, or intraocular triamcinolone injections. Inhibiting VEGF by antisense oligodeoxynucleotide or anti-VEGF mAb reduced or prevented iris neovascularization in animal models of CRVO. Previous clinical studies reported that intravitreal injections of 1.25 mg of bevacizumab were effective for patients with macular edema and ischemia from CRVO (Kriechbaum et al., 2008; Priglinger et al., 2007). Intravitreal bevacizumab in doses from 1 to 2.5 mg resulted in a significant decrease in macular edema and improved VA without short-term signs of toxicity in macular edema secondary to BRVO and CRVO (Byeon et al., 2007). In addition, early injection of anti-VEGF mAb seemed to change the unfavorable course of severe CRVO, and patients may have dramatic improvements in VA and clinical fundus appearance without collateral vessel formation. However, explanations for these recent observations are still necessary (Ferrara et al., 2007). Initial clinical series suggested that results of intravitreal bevacizumab are superior when used in individuals with non-ischemic CRVO when compared to those with ischemia, although ischemic CRVO has a favorable natural history (Kriechbaum et al., 2008; Priglinger et al., 2007). For BRVO, the indication for bevacizumab may depend on the amount of edema, and similar to patients with diabetes, multiple bevacizumab injections may be necessary.

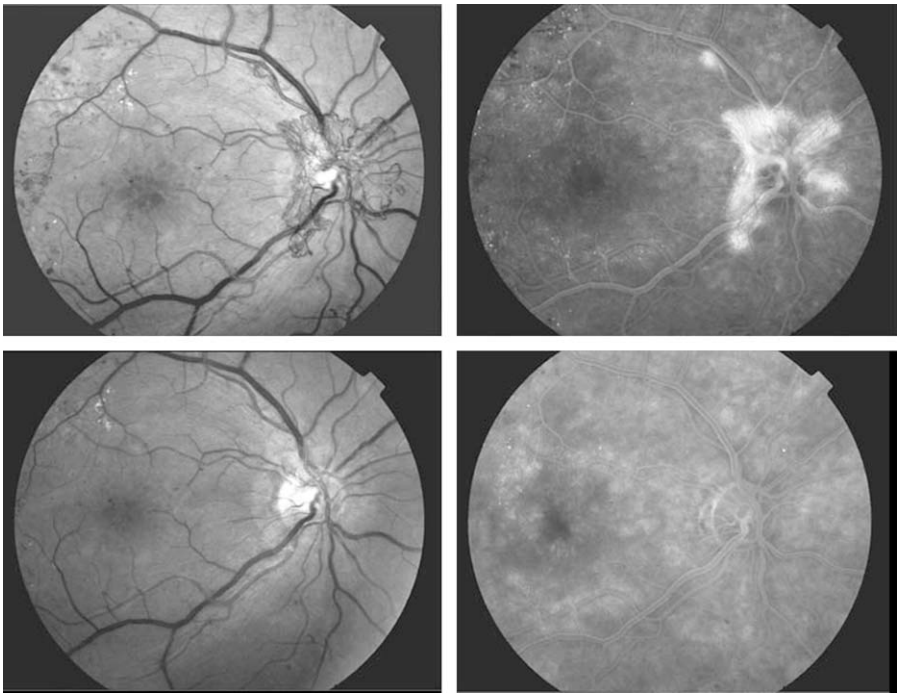

Fig. 4. A patient with PDR presents with neovascularization of the disc, shown in red-free photograph (left above) and fluorescein angiography (top right). Patient underwent intravitreal injection of bevacizumab, which led to regression of the new vessels, shown in the postoperative image (left below and right below). Reprinted from Friedlander et al., with permission.

Several uncontrolled clinical studies have reported the use of bevacizumab systemically and intravitreally to treat AMD. Initially, anti-VEGF mAb was evaluated in a human pilot study in which 5 mg/ml of bevacizumab was injected intravenously in patients with wet AMD. In that study, treatment with bevacizumab resulted in visual improvement and reduced retinal thickness evaluated by optical coherence tomography (OCT) examination of the CNV complex (Moshfeghi et al., 2006). Some phase 1 and 2 trials of intravenous bevacizumab are still under way. Furthermore, Nguyen et al. (2005) reported that systemic bevacizumab also suppresses CNV due to pathologic myopia. Other than systemic application, intraocular injection of bevacizumab at doses of 1.25–2.5 mg in repeated applications has yielded anatomic and functional improvement in patients with AMD as an off-label procedure over the past 3 years (Fig. 5) (Kaiser, 2006; Bashshur et al., 2008; Madhusudhana et al., 2007). However, there are still no established criteria of the frequency of administration for bevacizumab. Some clinicians follow therapeutic regimens that vary from monthly to every-3-month injections based on improved clinical signs such as VA improvement and resolution of subretinal, sub-RPE, or intraretinal fluid detected during biomicroscopic or OCT examination. Further clinical observations revealed non-responders with large CNV and initial bad VA (Lux et al., 2007). Other than AMD, small case series have reported that intravitreal bevacizumab is safe and potentially efficacious in eyes with subfoveal CNV secondary to pathologic myopia, angioid streaks, or chorioretinal inflammation or infection (Adán et al., 2007; Hernández-Rojas et al., 2007; Mandal et al., 2007; Prager et al., 2007; Rinaldi et al., 2007; Rosenfeld, 2007; Sakaguchi et al., 2007). Future investigations are warranted to elucidate the reasons young patients with CNV have a better response to bevacizumab.

The participation of VEGF in new vessel formation in ROP and neovascular glaucoma supports the idea of bevacizumab as a treatment option. Current ROP therapy relies on laser photocoagulation for threshold disease, whereas more advanced stages are treated with vitreoretinal surgery. Anti-VEGF treatments have been used successfully to inhibit neovascularization in oxygen-induced retinopathy in animals by inducing regression of iris and preretinal neovascularization in premature infants with severe ROP (Ozaki et al., 2000). However, this treatment did not completely and permanently inhibit angiogenesis in practice, and significant concerns have emerged about the effect of the drug on the normal physiologic development of both blood vessels and other tissue in preterm infants (Bhisitkul, 2006). Human studies have shown efficacy in the regression of new vessels, but no long-term results have been reported to date (Quiroz-Mercado et al., 2008). Despite recent reports describing the benefits of intravitreal injection of bevacizumab to treat ROP, possible adverse effects include worsening of retinal detachment (Honda et al., 2008). An important aspect regarding ROP is the intravitreal dose of bevacizumab used. Since newborns may be at higher risk of developing systemic complications from anti-VEGF drugs, smaller doses should be studied. For instance, 30 μg might be sufficient to inhibit retinal neovascularization in patients with DR. Future clinical trials with intravitreal bevacizumab for ROP should test very small concentrations in escalating doses.

For neovascular glaucoma, bevacizumab can be injected intravitreally or in the anterior chamber to promote regression of neovascularization. However, neovascularization may recur in 2 weeks (Yazdani et al., 2007). Ehlers et al. (2008) recently reported that the combination of intravitreal bevacizumab with panretinal photocoagulation resulted in a faster decrease of IOP than with panretinal photocoagulation alone. However, there has been no clear information on what approach would be more effective for treating neovascular glaucoma. More importantly, bevacizumab should not be considered a definitive therapy, it decreases IOP for only 2 weeks. Therefore, laser photocoagulation may still be necessary.

5.4. Anti-VEGF-fragment: ranibizumab

Ranibizumab is a humanized mAb-fragment designed to bind all isoforms of VEGF and as a result block vessel permeability and

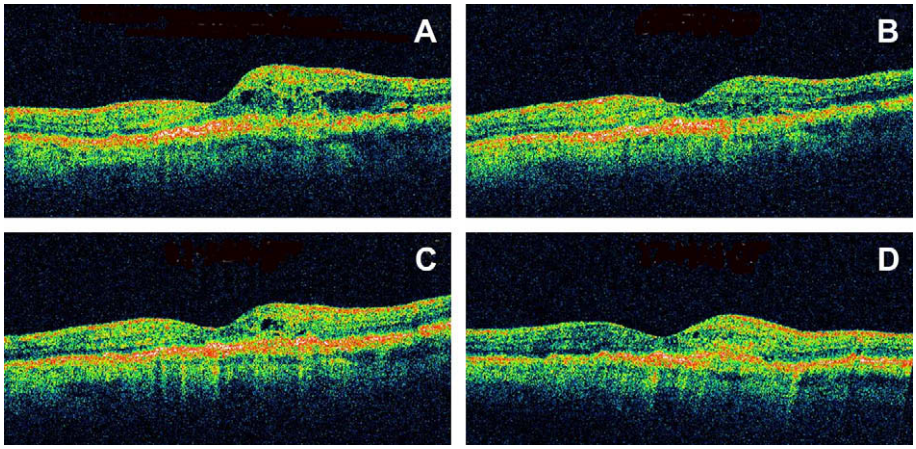

Fig. 5. A 77-year-old female patient presented with low vision due to occult CNV secondary to age-related macular degeneration; VA was 20/100. (A) Initial optical coherence tomography (OCT) examination revealed intraretinal fluid with deep RPE-choroid disorganization. Patient underwent one intravitreal injection of 1.25 mg bevacizumab. (B) Three-week post-injection OCT examination showed reduction of intraretinal fluid, VA was 20/80. Patient was submitted to a second injection of 1.25 mg bevacizumab 2 weeks later. (C) Two-week examination after the second bevacizumab injection, there is stabilization of the central macular thickness and VA of 20/80. Patient underwent a third bevacizumab injection 2 weeks later. (D) Four-week examination after the third bevacizumab injection, VA returned to 20/50 and there is disappearance of the intraretinal fluid. An area of deep hyperreflectivity remained corresponding to the fibrotic regressed CNV.

angiogenesis. Ranibizumab was derived from the humanized anti-VEGF Fab variant known as MB1.6 through a series of recombinant-DNA and phage-display selection steps produced in an *Escherichia coli* expression system. It contains five variable domain substitutions and one constant domain substitution at the C-terminus of the heavy chain compared with Fab-12. Ranibizumab binds to the receptor-binding site of all biologically active forms of VEGF-A and also penetrates all layers of the rabbit retina because of the small molecular size (48 kDa). Ranibizumab (10 mg/ml) is available in 0.3-ml vials and approved by the FDA for monthly intravitreal injections in patients with AMD. The binding of ranibizumab to VEGF-A prevents the interaction of VEGF-A with VEGFR1 and VEGFR2 on the surface of endothelial cells, reducing endothelial cell proliferation, vascular leakage, and new blood vessel formation (Ferrara et al., 2006).

Ranibizumab is an effective and safe antiangiogenic and anti-VEGF agent used to treat predominantly classic, minimally classic, and occult subfoveal CNV associated with wet AMD in two large phase 3, multicenter, randomized, double-masked, controlled pivotal trials, the MARINA and ANCHOR trials (Stone, 2006). The MARINA study (minimally classic occult trial of the anti-VEGF antibody ranibizumab in the treatment of neovascular AMD) randomized 716 subjects with minimally classic or occult CNV without classic subfoveal CNV to one of three treatment arms: monthly sham injections, monthly intravitreal injections of 0.3 mg of ranibizumab, or monthly intravitreal injections of 0.5 mg of ranibizumab. The MARINA met its primary end point at 1 year, with 95% and 96% of subjects in the 0.3-mg and 0.5-mg ranibizumab groups, respectively, losing less than 15 letters compared with 62% in the sham injection group ($P < 0.0001$). Ranibizumab maintained vision in about 95% of treated patients and improved vision in up to 40% of treated patients (Rosenfeld et al., 2006a,b). The ANCHOR (anti-VEGF antibody for the treatment of predominantly classic CNV in AMD) trial randomized 423 subjects with predominantly classic CNV to one of three treatment arms: verteporfin PDT with monthly sham ocular injections, monthly intravitreal injections of 0.3 mg of ranibizumab with a sham photodynamic therapy procedure, and monthly intravitreal injections of 0.5 mg of ranibizumab with a sham PDT procedure. The 1-year results from the ANCHOR study showed that 94% or more of patients treated with ranibizumab maintained VA compared to 64% of patients treated with PDT, while patients with ranibizumab had an average increase of 8.5 letters (0.3 mg ranibizumab) and 11.3 letters (0.5 mg ranibizumab) in mean VA (Kaiser et al., 2007). Currently, the mAb manufacturer, Genentech, is sponsoring various clinical trials to address concerns regarding the use of ranibizumab: the FOCUS trial (safety of ranibizumab in combination with PDT); the PIER study (efficacy of ranibizumab); the SAILOR study (safety of ranibizumab); and the PRONTO study (efficacy of ranibizumab based on OCT-guided treatment regimen) (Table 4). The PRONTO study protocol involving three monthly injections, followed by monthly examinations and retreatment if the retinal thickness increases over 100 μm or the VA decreases one or more lines. While several issues should be studied further, the initial clinical experience enables retina specialists to consider two treatment regimens for ranibizumab for CNV due to AMD: monthly injections for 2 years based on the MARINA/ANCHOR studies or the PRONTO-based therapy.

In addition to AMD, initial clinical studies reported a considerable benefit of ranibizumab for edematous and neovascular ocular diseases. Multiple sequenced injections of 0.5 mg ranibizumab may result in VA improvement and reduced retinal thickness in patients with DME due to DR or retinal vein occlusions (Nguyen et al., 2006; Campochiaro, 2007b). An in vitro study showed that ranibizumab restored VEGF-induced proliferation, migration, and decocalization of the tight junction proteins in retinal endothelial cells (Deissler et al., 2008). This broadens our knowledge regarding the action of anti-VEGF drugs in the treatment of inflammatory/edematous diseases. Investigations are needed to determine the precise role of ranibizumab for treating DME and retinal vein occlusion, especially the number of injections needed, the exact frequency of administration, and the optimal time of therapy initiation.

5.5. Novel and controversial issues in anti-VEGF therapy in ophthalmology

Several advantages are associated with the use of antiangiogenic therapies. First is the specificity to one particular aspect of the neovascularization process. A broad spectrum of antineovascular activity can be achieved if focusing specifically on one VEGF factor in ocular angiogenesis. Endothelial cells are optimal targets because they have a propensity not to develop resistant clones. Another advantage of this therapy is that antiangiogenic agents may inhibit ocular and CNV without affecting normal cellular vascularization. The rationale behind this hypothesis is supported by differences

Table 4
Summary of main clinical trials on Lucentis for neovascular age-related macular degeneration*.

Name of trial	Trial	Phase – design	Goal of study	Treatment regimen	Type of CNV
MARINA	Minimally Classic/Occult Trial of the Anti-VEGF Antibody Ranibizumab in the Treatment of Neovascular AMD	III	Efficacy	Monthly injections for 2 years	Minimally classic or occult
ANCHOR	ANti-VEGF Antibody for the Treatment of Predominantly Classic CHORoidal Neovascularization in AMD	III	Efficacy	Monthly injections for 2 years	Predominantly classic
PIER	Phase IIIb, Multicenter, Randomized, Double-Masked, Sham Injection-Controlled Study of the Efficacy and Safety of Ranibizumab	IIIb	Less frequent injections	Three injections followed by fixed 3-month injection interval for 2 years	All subtypes
SAILOR	Safety Assessment of Intravitreal Lucentis for AMD	IIIb	Safety of two doses	Three injections followed by an as-needed regimen for 1 year	All subtypes
PRONTO	Prospective OCT Study With Lucentis for Neovascular AMD	Interventional uncontrolled	Less frequent injections	Three injections followed by OCT-guided reinjection	All subtypes[a]
SUSTAIN	Study of Ranibizumab in Patients With Subfoveal Choroidal Neovascularization Secondary to Age-Related Macular Degeneration	IIIb	Safety and efficacy of an as-needed dosing regimen	Monthly injections for 1 year	All subtypes
EXCITE	Efficacy and Safety of Ranibizumab in Patients With Subfoveal CNV Secondary to AMD	III	Efficacy two different designs	Three injections followed by comparison monthly vs. every-3-month intervals for 2 years	All subtypes
PROTECT	Open-Label, Multicenter, Phase II Study Assessing the Effects of Same Day Administration of Ranibizumab (Lucentis) and Verteprofin PDT	I/II	Safety of combination with Visudyne PDT injected on the same day[b]	Lucentis in combination with PDT compared to PDT alone	Predominantly classic
FOCUS	RhuFab V2 Ocular Treatment Combining the Use of Visudyne(R) to Evaluate Safety	I/II	Efficacy of combination with Visudyne PDT	Lucentis in combination with PDT compared to PDT alone	Predominantly classic

* As of April 2008.
[a] If predominantly classic CNV patient must have had prior PDT.
[b] Formulation of Lucentis as used in the two registration trials MARINA and ANCHOR.

between the relative quiescence of normal endothelial cells vs. the actively proliferating and migrating endothelial cells and the angiogenic network.

Because anti-VEGF mAbs are in widespread use, many issues still need to be addressed in more detail, specifically, the appropriate dose of anti-VEGF mAbs for ocular neovascularization, the frequency of use with which the drug will be most effective against ocular neovascularization, the time to use the drug to achieve the best results, the mechanisms of failure of antiangiogenic treatments (e.g., resistance), the role of combined therapies involving anti-VEGF mAbs, and the differences in efficacy between bevacizumab and ranibizumab.

The standard dose of 0.5 mg of ranibizumab has been introduced after a few pre-clinical experiments in primates, while Rosenfeld et al. (2005) established 1.25- to 2.5-mg doses of bevacizumab based on the theoretical half-life and binding capacity of the full-length mAbs and comparisons of the dose with ranibizumab. Because ranibizumab has a molecular weight of about one-third that of bevacizumab, the equivalent number of bevacizumab molecules to obtain a similar effect to that of ranibizumab has ranged in doses of 0.9–1.5 mg. However, there may be some evidence that extreme doses might be effective to treat ocular angiogenesis. Avery et al. (2006) reported that doses as low as 30 μg of bevacizumab may inhibit retinal neovascularization in PDR, while Klettner and Roider (2008) showed in vitro that only a fraction of ranibizumab and bevacizumab mAbs was necessary for VEGF neutralization; such small doses may decrease the risk of toxicity. However, low doses could mean repeated injections, and in diseases in which the anti-VEGF mAbs must reach the choroid, very small doses are less likely to be effective. However, higher doses of intravitreal bevacizumab or ranibizumab such as 5 or 10 mg in theory may decrease the necessity for repeated injections and increase the penetration of mAbs. This several-fold increase in dose may overcome barriers to diffusion, allowing a reasonable amount of this agent to diffuse into the retina and produce a biologic effect. However, increased systemic absorption may be expected leading to an increased risk of complications such as high blood pressure or hemorrhage. In addition, earlier principles of mAb application highlighted that with excessive doses to block an mAb activity the opposite effect may occur. Oncologic experience has shown that acute administration of bevacizumab reduces circulating endothelial cells but increases the fraction of tumoral endothelial cells with pericyte coverage in patients with colorectal cancer; such coverage delays final reduction of the angiogenic network.

The best approach for the use of anti-VEGF in posterior segment diseases is through intravitreal injections. However, there may be better regimens for intravitreal administration in terms of frequency of use to make the drug more effective. However, there are still no established criteria regarding the frequency of administration for bevacizumab. Clinicians use drug injections in regimens varying from monthly to every 3 months based on the signs of clinical efficacy such as visual improvement and resolution of subretinal, sub-RPE, or intraretinal fluid on biomicroscopic or OCT examination. Currently, a loading dose of three injections is recommended for ranibizumab and bevacizumab based on the PRONTO Study, followed by injections guided by OCT, angiographic, or VA worsening. However, such a loading dose could be increased to weekly or biweekly injections to promote a greater reduction in tissue and network retinal VEGF levels or increased mAb penetration. The rationale is that after a few days the retinal concentrations of anti-VEGF decrease markedly and disappear after 3 weeks. However, the high frequency of injections may increase the risk of infection or other injection-related problems. The issues of frequency of injections at different intervals of administration of bevacizumab or ranibizumab are unknown and have not been investigated. In contrast, less frequent injections may allow disease progression or even expose the macula to chronic macular edema, which may damage the photoreceptors permanently. Maximal antiangiogenic activity typically requires prolonged exposure to mAbs. In addition, a novel approach could be the use of increasing doses of mAbs anti-VEGF, which was suggested recently for trastuzumab or alemtuzumab in cancer therapy. In addition, for inflammatory diseases, infliximab is administered intravenously by repeated infusions at 2, 6, and every 8 weeks afterward. This

approach aims to decrease side effects, determine the optimal dose for successful disease control for each patient, and decrease the risk of toxicity. After initial infusion, the infusions are repeated. The usual starting dose is 3 mg/kg and can increase up to a maximum of 10 mg/kg.

Another important aspect involving anti-VEGF mAbs is the timing of the start of therapy. To treat CNV secondary to AMD, anti-VEGF mAbs should be initiated immediately after diagnosis, since VEGF dependence has been reported in endothelial cells of newly formed but not mature vessels. Indeed, coverage by pericytes appears to be a key event resulting in endothelial loss of VEGF dependence. However, for DME, PDR, or even uveitis, the optimal moment to start anti-VEGF mAbs needs clarification. In DME, early VEGF inhibition may prevent macular edema damage. However, VEGF may be only a partial mediator of a complex edematous cascade in that disease. Regarding PDR, preliminary clinical experience showed that intravitreal bevacizumab injections can promote only temporary inhibition of proliferation and, therefore, laser photocoagulation remains an important initial approach. Thus, future studies should evaluate when anti-VEGF should be injected in patients with PDR: as soon as it is diagnosed, in cases refractory to laser photocoagulation, before vitrectomy, or in cases of neovascular glaucoma.

Recent clinical experience indicated that there may be resistance and tolerance to both ranibizumab and bevacizumab after multiple intravitreal injections for diseases such as CNV. However, the mechanisms of resistance to these mAbs are not known completely and may involve host and cellular factors affecting the response. Induction of apoptosis, antibody-dependent cell cytotoxicity, pharmacokinetics, microenvironmental alterations, the Fc receptor polymorphism, mutations at the antibody-binding sites, and complement-mediated cell death are some of the proposed mechanisms. If the main mechanism involves binding of natural antibodies to the Fc portion, this phenomenon would probably be more common with bevacizumab than ranibizumab. For example, mutations conferring resistance to neutralization with mAbs can be located outside or inside the antibody-binding site, and they may be controlled by genetic variability and the presence of cytokine-receptor polymorphisms; such knowledge would be extremely helpful because it could predict clinical failures. Experimental studies from the oncology field have shown that chronic VEGF-A inhibition may shift and increase the production of $VEGF_{165b}$, which is an angiogenic isoform of VEGF-A. In addition, lack of efficacy of bevacizumab may be explained by cellular toxicity of chemical components of the commercially available solution. The 100-mg vial of bevacizumab contains salts such as 240 mg α,α-trehalose dihydrate, 23.2 mg sodium phosphate (monobasic, monohydrate), 4.8 mg sodium phosphate (dibasic, anhydrous), or emulsifiers such as 1.6 mg polysorbate 20. Polysorbate severely damages retinal cells in doses of 2 mg/ml (Valamanesh et al., 2007). Moreover, bevacizumab is available in a slightly acidic solution (pH 6.2), and neuronal tissues such as the retina are sensitive to small acid–base changes. Our group is investigating in cell culture the retinal biocompatibility with different salts, emulsifiers, changes in pH, and osmolarity to clarify these issues. In addition to these improvements, potential clinical approaches to overcome resistance to anti-VEGF mAbs or other mAbs include the use of a biologic combination of new mAbs for therapy, changing one anti-VEGF mAb, e.g., bevacizumab, for other, e.g., ranibizumab; the use of alternate injections of bevacizumab and ranibizumab; or even combinations of both bevacizumab or ranibizumab.

The role of combining anti-VEGF mAb to other mAbs therapies is an important and controversial aspect in ocular therapeutics. Angiogenesis is a complex phenomenon and can be induced by a number of cytokines. For example, different pathways of angiogenesis depend on different biochemical factors and cascades, including vascular cell integrins, and bFGF, in addition to VEGF. VEGF-driven angiogenesis seems to be a major factor in ocular neovascularization. However, not every patient responds fully to anti-VEGF agents. Therefore, other factors may stimulate proliferation of vascular endothelial cells. In this context, inhibition of VEGF with mAbs would represent the basic approach and the addition of other agents to target other factors would result in a combined therapy. The efficacy of those combinations would depend on the type of angiogenesis, patient biology, age, or more complex mechanisms. Some of the recently proposed combined therapies for CNV in small cases series include intravitreal bevacizumab/ranibizumab plus dexamethasone plus PDT; bevacizumab plus triamcinolone; and intravitreal bevacizumab plus gas plus tissue plasminogen activator; PDT and plus ranibizumab/bevacizumab (Meyer et al., in press). Those clinical studies have showed that combinations may potentiate the antiangiogenic effect. However, few specific recommendations can be made since these studies were non-comparative, retrospective, and case series. Combination therapeutic mAbs may have different effects: synergizing, potentiating, or inhibiting functioning. Nevertheless, one reasonable approach for classic and occult CNV is to start with monthly intravitreal anti-VEGF mAb therapy, and once a refractory state is diagnosed, the addition of an intravitreal injection of a steroid such as triamcinolone or dexamethasone should be considered. Combined therapies with other mAbs such as anti-TNF mAbs; anti-EGF mAbs; or anti-platelet-derived growth factor (PDGF) also may have a substantial effect.

An important issue in antiangiogenic therapy with anti-VEGF mAbs is to determine which agent, ranibizumab or bevacizumab, is more effective. The main differences between these two agents are shown in Table 5. A potential advantage of bevacizumab over ranibizumab is its predicted longer half-life in the eye found in animal studies. However, ranibizumab has been genetically engineered, resulting in a 140-fold higher binding affinity of a single site compared to bevacizumab. Therefore, more bevacizumab would be needed to obtain a similar effect. Important information regarding their efficacy to inhibit VEGF arises from in vitro studies, as reported by Klettner and Roider (2008). These authors have shown that the clinical dose of bevacizumab is as effective as ranibizumab, but when diluted, ranibizumab was more effective than bevacizumab. Reduced immunogenicity also seemed to be an advantage of Fab molecules such as ranibizumab over full-length mAbs like bevacizumab. Fab molecules were believed to have significantly reduced risk from innate immune responses due to the absence of the constant antibody region (Fc). Early safety studies of ranibizumab reported that no antibodies against ranibizumab were detected. However, an increased risk of inflammation led to reformulation of ranibizumab. The full-length mAb bevacizumab, by virtue of its Fc domain, is believed to be more immunogenic than ranibizumab, but the immunogenicity profile of bevacizumab has not been examined in a clinical trial. Differences in the efficacy of both anti-VEGF will be investigated by a National Eye Institute-funded clinical trial that started in February 2008, named (Comparison of AMD Treatments Trials, CATT). This trial will randomly examine monthly injections of either mAb agents for CNV in AMD.

5.6. Ocular and systemic risks with anti-VEGF therapy in ophthalmology

5.6.1. Intraocular safety

In contrast to the usual preliminary evaluation of any drug in animals before clinical investigation, bevacizumab for ocular therapy was tested first in humans. Due to the demands for the clinical application of bevacizumab, many laboratory and animal studies evaluated the biocompatibility and safety of this drug in

Table 5
Differences between mAbs anti-VEGFs Ranibizumab and Bevacizumab.

	Bevacizumab	Ranibizumab
Commerical name	Avastin	Lucentis
Molecular size (kDa)	149	48
Fabs constitution	Residues involved in six CDRs and several framework residues changed to murine counterparts	Five variable domain and one constant domain substitution compared with Fab-12
VEGF affinity enhancement	No	14- to 100-fold
Technique for affinity enhancment	No	Phage-display
Type of mAbs	Humanized full-length mAb IgG1	Humanized Fab fragment
First year of production	1996	1998
Technique of production	Direct Humanization from Fab-12 of murine Mab A.4.6.1	Recombinant-DNA and phage-display selection of Humanized A.4.6.1
Route of administration in humans	Endovenous and intravitreal	Intravitreal
Dose for intravitreal injection (mg) for ARMD	1.25–2.5	0.5
Dose for intravitreal injection (mg) for DR	0.03–2.5	0.5
Intravitreal frequency regimen of intravitreal injection for ARMD	Variable from monthly to longer periods	Monthly
Intravitreal half-lives after intravitreal injections (days)	4.9[a]	2.8–3.2[b]
Systemic half-lives after intravenous application in primates (days)	21	<1
Systemic half-lives after intravitreal application in primates (days)	6.9[a]	3.5[b]
Peak serum concentration after intravitreal injection (ng/ml)	3000	0–3 (humans); 150 (small primates)
FDA approval indication	Cancer Colon	Age-related macular degeneration
Level of evidence for ARMD	Retrospective non-randomized clinical series	Prospetive randomized controlled clinical trial
Level of evidence for non-ARMD neovascular eye diseases	Retrospective non-randomized clinical series	Retrospective non-randomized small clinical series
Risk of immunogenic response	Yes	No
Approximate cost per intravitreal injection (US$)	~30	~1950

[a] 1.25 mg intravitreal bevacizumab.
[b] 0.5 mg ranibizumab.

ophthalmology. Consecutive experimental investigations in rats, rabbits, and primates showed that intravitreal bevacizumab at different concentrations up to 3 mg/ml did not cause any functional or morphologic retinal toxicity (Bakri et al., 2007b; Inan et al., 2007; Manzano et al., 2006; Shahar et al., 2006). In vitro cellular assays examining exposure to various concentrations of bevacizumab (0.08–1 mg/ml) have shown little toxic effects on ganglion cells, neuroretinal cells, and RPE cells (Iriyama et al., 2007; Lüke et al., 2006; Luthra et al., 2006). However, a few recent animal studies reported some signs of retinal damage after intravitreal bevacizumab, such as choriocapillaris changes in primates, intraocular inflammation in rabbits, and mitochondrial abnormalities in the rabbit retina (Inan et al., 2007; Manzano et al., 2006; Peters et al., 2007; Shahar et al., 2006).

Regarding the pharmacokinetics of bevacizumab, the mAbs penetrated the full thickness of retinal tissue in 24 h, and the concentrations gradually decreased to undetectable levels by 4 weeks in rabbits (Bakri et al., 2007a,b). The half-life of the humanized mAbs injected intravitreally is around 5 days. Experiments in monkeys showed bevacizumab immunoreactivity in the choroid and in the inner retinal layers as early as 1 day after the injection, thereafter spreading to the outer layers and choroid within 7 days (Heiduschka et al., 2007).

The pre-clinical safety of ranibizumab was evaluated in primate eyes; a 0.5-mg injection of the mAb-fragment promoted reduced leakage in cases with CNV with no retinal toxicity (Krzystolik et al., 2002). The mAb fragment showed great biocompatibility in vitro with RPE and neuroretinal cells (Spitzer et al., 2007). The pharmacologic and clinical differences between bevacizumab and ranibizumab are listed in Table 5 (Bakri et al., 2007b). Such pre-clinical data enable widespread clinical use of ranibizumab for wet AMD therapy and other edematous and neovascular retinal conditions.

Intraocular bevacizumab and ranibizumab injections have few clinically relevant ocular side effects. In contrast to the crystalline steroid drug triamcinolone acetonide, intravitreal bevacizumab does not induce glaucoma or cataract progression. Further clinical experience with intravitreal bevacizumab revealed few sporadic cases of uveitis, vitreous hemorrhage, RPE tears, or endophthalmitis (Chan et al., 2007; Rodrigues et al., 2007; Rosenfeld et al., 2005). Rosenfeld et al. (2005) proposed the maximum tolerated dose of ranibizumab to be 0.5 mg, since doses above 1 mg promoted clinically relevant intraocular inflammation. In another clinical investigation, intravitreal ranibizumab induced few severe complications such as endophthalmitis, uveitis, and vitreitis in the fellow eye. Minor ocular events included conjunctival bleeding, ocular pain, and floaters (Rosenfeld et al., 2006a). We recently analyzed 17 cases of ocular and systemic hemorrhage after intravitreal bevacizumab injection; the ocular hemorrhagic events included vitreous hemorrhage, intraretinal damage, and subretinal damage. These events probably occurred due to molecular changes induced by bevacizumab including a decrease in the VEGF-dependent coagulation tissue factor, platelet damage, or injury of normal endothelial vascular cells (unpublished data). The Pan-American Collaborative Retina Study Group (PACORES) evaluated the ocular side effects of over 4303 injections of bevacizumab in 1773 patients and reported ocular complications including seven (0.16%) cases of bacterial endophthalmitis, seven (0.16%) cases of tractional retinal detachments, four (0.09%) cases of uveitis, and one case (0.02%) of rhegmatogenous retinal detachment and another of vitreous hemorrhage. The authors claimed that intravitreal injections of bevacizumab generally are safe for treating vitreoretinal diseases (Wu et al., 2008). In 2007, the National Eye Institute (Bethesda, MD, USA) funded a head-to-head comparison of ranibizumab vs. bevacizumab to treat advanced AMD to elucidate safety differences between the two mAbs (http://www.nei.nih.gov/news/statements/amd_therapy.asp).

The constitutive expression of the molecule in normal quiescent ocular and other tissues suggests unknown but potentially

important functions. It has been speculated that VEGF blockage may cause increased apoptosis among ganglion cells, photoreceptors, or even choroidal tissue. In vitro experiments suggest that VEGF plays a role in photoreceptor differentiation and may contribute to photoreceptor survival. Moreover, basal production of VEGF by the RPE may maintain certain capillary beds, as suggested for the choroid plexus. If a similar mechanism is operative between the RPE and the choroidal capillaries, VEGF inhibition could lead to choroidal atrophy. Therefore, both systemic and local ocular inhibition of VEGF could have serious side effects. However, no clinical evidence for such neuroretinal damage has been observed after chronic anti-VEGF therapy.

5.6.2. Systemic risks of anti-VEGF mAbs

One recent controversy concerns systemic absorption of bevacizumab after intravitreal injection. Bakri et al. (2007a,b) examined the pharmacokinetics of intravitreal injection of 1.25 mg bevacizumab in rabbits and found that a maximum serum concentration of 3.3 μg/ml was achieved 8 days after intravitreal injection, decreasing below 1 μg/ml after 29 days. However, intraocular ranibizumab injection resulted in lower serum drug levels. The maximum serum concentration observed after bilateral intravitreal injection was 150 ng/ml. In monkeys, the vitreous half-life of ranibizumab has been 3 days and after intravitreal injection of 0.5 mg, the maximum serum level observed was 150 ng/ml, with a serum half-life of 3.5 days (Gardreault et al., 2005). In human studies, the mean serum concentration of ranibizumab 1 h after intravitreal administration (0.3 mg) was 1.35 ng/ml and, after 28 days, serum concentrations were below 0.300 ng/ml in 96% of the patients (Bhisitkul, 2006). Such levels of serum ranibizumab are below the approximate 10 ng/ml threshold estimated to affect VEGF-A-related activity in humans. Further clinical studies should help determine if higher penetration of bevacizumab into the circulation could be more harmful to patients with ocular diseases compared to ranibizumab.

Information about the systemic risks of endogenous anti-VEGF therapy obtained from clinical and experimental studies showed that the main factor for complications is the interference with human VEGF biology. The most frequent complications after systemic anti-VEGF bevacizumab for cancer therapy include hypertension, proteinuria, hemorrhage, thromboembolism, impaired wound healing, gastrointestinal perforation, and leukoencephalopathy (Verheul and Pinedo, 2007). Hypertension may affect up to 30% of patients using systemic bevacizumab, and the etiology may involve nitric oxide blockade secondary to VEGF inhibition (Shen, 1999). Hemorrhage in mucocutaneous membranes occurs in 20–40% and could be related to the VEGF-dependent endothelial maintenance and interference with the coagulation cascade and tissue factor. Moreover, a slight increase in the incidence of arterial thromboembolism has been found in patients with cancer treated with endogenous bevacizumab compared to the control group. In addition, bevacizumab may affect surgical wound healing or suppress angiogenesis of the female reproductive tract. Future studies are important to investigate complications after systemic use of bevacizumab and these potential toxic effects may limit the application of this drug for therapy of ocular diseases.

The incidence of non-ocular adverse events after intravitreal bevacizumab was initially evaluated by an Internet survey. Physician-reported events included transient ischemic attack, blood pressure elevation, cerebrovascular accident, and death (Fung et al., 2006). However, recent case reports showed complications such as changes in arterial blood pressure or metrorrhagia (Rodrigues et al., 2007). The PACORES group also addressed the systemic safety of over 4303 injections in 1773 patients undergoing bevacizumab therapy of different diseases. The investigators found systemic side effects in 18 (1.5%) patients. These included seven (0.59%) cases of elevated systemic blood pressure, six (0.5%) cases of cerebrovascular accidents, five (0.4%) cases of myocardial infarctions, two (0.17%) cases of iliac artery aneurysms, two (0.17%) cases of toe amputations, and five (0.4%) deaths (Wu et al., 2008). A survey that investigated the risks of intravitreal bevacizumab reported only increased systemic blood pressure (Wong et al., 2008).

Regarding the safety of ranibizumab, pre-clinical studies of an anti-VEGF recombinant humanized mAb closely related to ranibizumab involving young adult cynomolgus monkeys showed physeal dysplasia after intravenous doses of the antibody at 2 mg/kg. At doses of 10 mg/kg, uterine and ovarian weights decreased and the corpora lutea were damaged. Partial recovery of these changes was seen 4 weeks after treatment stopped. These results suggest that VEGF inhibition may expose normal organs to severe damage and, therefore, care should be taken.

Overall, controlled phase 3 trials including the 2-year MARINA and ANCHOR studies of intravitreal ranibizumab reported initial safe application of these drugs without an increased incidence of systemic adverse events such as myocardial infarction and stroke (Rosenfeld et al., 2006b). However, a recent report that analyzed the combined results from the MARINA and ANCHOR trials showed a higher incidence of non-ocular hemorrhage after the use of intravitreal ranibizumab. The rates of thromboembolic events were also higher in the 0.5 mg ranibizumab treated groups (Gillies and Wong, 2007). The SAILOR study investigated the risks of ranibizumab in patients with AMD. An interim analysis of the SAILOR study completed in early 2008 showed an increased risk of stroke in patients who received the 0.5-mg dose. The 1-year results are expected soon, and until they are available no final conclusions can be drawn on the systemic safety of ranibizumab.

Based on those preliminary experimental and clinical data, the use of anti-VEGF mAb in selected groups of patients may be contraindicated: breast-feeding women, patients with history of coronary heart attack during the previous 3 weeks, children, patients with a history of recent gastrointestinal surgery, and patients with current uncontrolled hemorrhagic disease (e.g., epitasis). In specific patients, the drug could be carefully indicated under strict conditions: patients with uncontrolled high blood pressure, patients with a history of stroke, and patients with active aneurysms. Regarding the use of anticoagulants, there is no correlation between the use of aspirin and non-ocular hemorrhages even with systemic application of bevacizumab in cancer therapy (Horn and Hoerauf, 2008).

6. Therapeutic mAbs against other pro-angiogenic and proinflammatory soluble growth factors and cytokines

Additional growth factors involved in the homeostasis and pathologic processes of ocular cells/tissues include PDGF, epidermal growth factor (EGF), fibroblast growth factor (FGF), insulin-like growth factor (IGF), hepatocyte growth factor (HGF), and the transforming growth factor (TGF) families.

6.1. mAbs anti-TGF-beta

The TGF-beta superfamily is a powerful stimulator of scarring and wound healing processes through stimulation of fibroblasts in many organs including the eye. In ocular tissues and diseases, TGF-beta2 is the predominant isoform involved in pathological and scarring processes of the cornea, retina, trabecular meshwork, and conjunctiva (Pfeffer et al., 1994). TGF-beta may be a key molecule in corneal wound healing after excimer laser photorefractive keratectomy (Tuli et al., 2006). Some investigators have suggested that

TGF-beta is involved in the scarring process of primary retinal detachment repair in PVR (Kon et al., 1999). TGF-beta also plays a role in the genesis of glaucoma by several mechanisms involving the trabecular meshwork tissue, aqueous outflow, and subconjunctival scarring response after glaucoma filtration surgery. Studies of the effects of members of the TGF-beta family revealed that this factor causes cataractous changes in lens epithelial cultures, with TGF-beta2 having a more powerful effect than TGF-beta1. The ocular media contain molecules that inhibit TGF-beta activity and block its cataractogenic effects on lens cells. Conditions favoring the release of active TGF-beta include low pH and overexpression of proteases. The role of an anti-TGF-beta2 human mAb, lerdelimumab (CAT-152, Cambridge Antibody Technology; Cambridge, UK) has been studied for glaucoma filtration surgery, prevention of cataract development, postoperative posterior capsule opacification (PCO), and PVR. To prevent and treat PVR, lerdelimumab was studied in phase 1 and 2 trials. However, the study was stopped because an unusual retinal lesion was observed in 30% of those participating in the trial (Mead et al., 2003). Preclinical studies showed the efficacy of anti-TGF-beta mAb in reducing conjunctival scarring in vitro and in vivo. Results of a randomized controlled study showed no benefit of lerdelimumab as a postoperative agent to prevent scarring after glaucoma surgery compared to 5-fluorouracil and placebo (CAT-152 0102 Study Group, 2007). The manufacturer will focus their resources on studies of other types of mAbs for non-ophthalmologic purposes. Therefore, little information regarding the application of this anti-TGF drug is expected in the near future (www.cambridgeantibody.com./home/news_and_resources/news_archive/2005/cambridge_antibody_technology/announces4).

6.2. mAb anti-PDGF

PDGF consists of two homologous polypeptides A and B to generate five recognized isoforms that activate the cellular responses to play important roles in embryonic development and cell proliferation of fibroblasts and angiogenesis. In the eye, PDGF is a well-characterized stimulatory factor in the migration of RPE and retinal glial cells, which may promote severe proliferative retinopathy related to retinal detachment and PDR, while in corneal epithelial cells both fibroblast migration and collagen contraction have been stimulated in a dose-dependent manner by PDGF-AB (Seo et al., 2000; Imanishi et al., 2000). Combining the PDGF antagonists and blockage of VEGFs has been proposed as a valid approach to treat ocular neovascularization. Within the neovascular network, vascular endothelial cells release PDGF-B, which in turn induce recruitment, proliferation, and survival of pericytes, glial cells, and RPE cells (Campochiaro and Glaser, 1985). Newly established pericytes along with retinal cells provide survival signals for endothelial cells, and more importantly, pericytes may promote the scarring process following CNV (Bergers et al., 2003). Mural cell recruitment to the growing endothelial tube is regulated by PDGF-B signaling; interference with this pathway causes disruption of endothelial cell–mural cell interactions and loss of mural cells. Therefore, antagonists of PDGFs with or without VEGF antagonists may reduce scarring and neovascularization. Moreover, inhibition of both VEGF-A and PDGF-B signaling may be more effective than blocking VEGF-A alone in causing vessel regression in multiple models of neovascular growth (Campochiaro, 2007a,b,c). A therapeutic fully human anti-PDGF type D mAb named CR002 (CuraGen, Branford, CT, USA) ameliorates tubulointerstitial fibrosis after experimental glomerulonephritis, whereas a fully human-mAb anti-PDGFR-alpha 3g3 (ImClone Systems Inc., New York, USA) blocks PDGF-AA and PDGF-BB ligands from binding to the PDGF receptor in tumors such as glioblastoma and leiomyosarcoma (Loizos et al., 2005). The company has not started early clinical trials with anti-PDGF for wet AMD. While this drug theoretically may reduce the chances of fibrosis development in CNV, it is too early to assume its clinical efficacy.

6.3. mAb anti-EGF

Human EGF is a protein with 53 amino acid residues and three intramolecular disulfide bonds that may play important functions in the regulation of cell growth, proliferation, and differentiation in various tissues. Despite the great importance of VEGF in the targeted stimulus of angiogenesis, EGFR also participates in the regulation of the cellular events of vascular endothelial cell proliferation, repair, and survival. The binding of the EGFR receptor starts many stimuli to cell signaling pathways, thereby promoting up-regulation of pro-angiogenic cytokines including VEGF and matrix metalloproteinase that cause the neovascular network to grow. EGF is present in a variety of rat ocular and periocular tissues including ocular surface tissues as the lens, chorioretina, cornea, tear fluids, extraorbital lacrimal glands, intraorbital lacrimal glands, Harderian gland, and conjunctiva. Exogenous EGF stimulates mitogenesis of the corneal and lens epithelium during wound healing and PCO after cataract surgery (Watanabe et al., 1993). EGF also may participate in the pathophysiology of PVR associated with rhegmatogenous retinal detachment and DR, as well as ocular melanoma (Heidenkummer and Kampik, 1991).

Cetuximab (Erbitux®; ImClone Systems/Bristol-Myers Squibb, New York, USA) is a recombinant chimeric mAb specifically targeting the extracellular domain of the human EGFR; in combination with irinotecan the new mAb improved control of metastatic colorectal cancer in phase 2 and 3 studies. Cetuximab competes with an endogenous ligand to bind to the extracellular domain of EGFR, although other mechanisms of action include down-regulation of cell-surface receptors and thereby promotion of apoptosis, inhibition of the production of angiogenic factors, and mediation of antibody-dependent cell-mediated cytotoxicity. Other EGFR antibodies that have undergone phase 1 and 2 studies in oncology are the murine ER-HR3 and panitumumab (Abgenix/Amgen Malcolm Ranson, Thousand Oaks, CA, USA), a fully human IgG2 anti-EGFR mAb. Experimental and clinical data on the safety and efficacy of EGF inhibitors for ophthalmic diseases showed that systemic administration of an EGF receptor tyrosine kinase inhibitor may decrease epithelial proliferation and stratification in response to corneal injury and promote regression of new corneal vessels in an animal model (Nakamura et al., 2001). These preclinical data provide the rationale for anti-EGF use in the treatment of various ophthalmic diseases to control ocular melanoma as adjuvant therapy to radiotherapy, to prevent or treat ocular angiogenesis or PVR, to maintain corneal transparency during wound healing, and to prevention of PCO. For such indications, these agents can be administered with intracamerular or intravitreal cetuximab. However, further studies are needed because there are only weak cause–effect relationships, and mediators other than EGF may play a more important role in these entities.

The antiangiogenic activity of EGFR-targeting drugs could be generated by a dual effect: inhibition of pro-angiogenic factors, such as VEGF, and a direct effect on the intratumor endothelial cells. Indeed, combining mAbs that interfere with the VEGF and EGF receptors has had a substantial effect in pre-clinical cancer models to optimize endothelial cell targeting with a powerful anti-angiogenic effect. Since EGF in conjunction with VEGF may exert biologic effects directly or indirectly on tumor growth angiogenesis through activation of their specific downstream signaling, a similar combination approach may be evaluated to treat ophthalmic proliferative and oncologic entities such as ocular melanoma,

prevent or treat ocular angiogenesis, and maintain corneal transparency during wound healing. A trial on the use of cetuximab plus anti-VEGF mAbs such as ranibizumab or bevacizumab compared with cetuximab or other anti-EGF mAbs such as panitumumab alone may be useful for ocular neovascularization. If the safety of the anti-EGF mAbs is demonstrated, additional trials could be performed to evaluate combining other mAbs. However, such a trial should be preceded by evaluation of toxicity and pharmacokinetics if they are locally administered.

Clinical signs of cetuximab toxicity to the eyelid and conjunctiva such as ectropion and conjunctivitis have been reported after systemic administration of the anti-EGF mAb (Melichar and Nemcova, 2007). The pathogenesis of extraocular complications by cetuximab may involve targeting of the EGFR-expressing cells of the meibomian glands to consequently lead to altered secretive function.

6.4. mAb anti-HGF

A few additional cytokines such as HGF, bFGF, and IGF may be targeted in the therapy of ocular diseases in the future. HGF is a multipotential cytokine that possesses multiple biological mitogenic, morphogenic, anti-apoptotic, and angiogenic activities in ocular health and disease. Besides fibroblasts as the main source, other cells that produce HGF/SF (scatter factor) include, e.g., vascular smooth muscle cells, endothelium, glial cells, macrophages, activated T-lymphocytes, and various tumor cells. In addition, HGF can be produced by stromal keratocytes and, along with other growth factors, may have a role in corneal development, homeostasis, and repair of epithelial erosions (Wilson et al., 1999). In corneal wound healing, HGF/SF may be a key player in stromal fibroblast modulation of epithelial and endothelial behavior during the repair activities after corneal injury. In addition, the vitreous levels of HGF/SF are elevated in PVR and PDR in which HGF is a major angiogenic factor. HGF/SF may play a role in corneal vascularization but as yet there have been no studies on the distribution and function of the cytokine in corneal and chorioretinal diseases associated with new vessel formation. Tens to hundreds of nanogram levels of HGF/SF injected into rat corneas induced corneal neovascularization, and, therefore, may be considered as a potential inducer of angiogenesis. Finally, HGF may be involved in metastasis of uveal melanoma, because HGF participates actively in the formation of the vascular channels in tumor cells.

L2G7 (no registered brand by 2007, Galaxy Biotech, Mountain View, CA, USA) is a novel anti-HGF humanized mAb generated by hybridoma technology that blocks binding of HGF to the receptor named Met, which can have profound anti-tumor effects even within the central nervous system, although for ocular diseases anti-HGF mAb still remains to be studied. In contrast to various other factors, HGF up-regulation in ocular melanoma by melanocytes has been established, which makes this cytokine a favorable goal for anti-HGF therapy to decrease the risk of hematogenous spread of ocular melanoma. Disseminating ocular melanoma cells are particularly responsive to HGF/SF as a mitogen and, therefore, the growth factor may be a specific signal for metastatic behavior. For corneal stromal disease, blocking the cytokine with an anti-HGF mAb may assist in modulating severe stromal injury. Regarding PVR and PDR, L2G7 or future newer anti-HGF mAbs may be considered for clinical use, especially with other mAbs against VEGF, TNF, and EGF, although this is just theoretical and must be proven with further experimentation and clinical data.

6.5. mAb anti-bFGF

FGFs are a family of heparin-binding growth factors involved in wound healing and embryonic development. The basic-FGF form, also referred to as b-FGF, may be a more potent angiogenic factor than VEGF or PDGF. In the eye, FGF is localized within the lacrimal gland, retina, lens, photoreceptors, aqueous humor, vitreous, and corneal epithelium. In both retina and RPE cells, FGF induces changes in cellular proliferation and in vivo angiogenesis. FGF also has been implicated in the proliferation of lens epithelial cells into fiber cells and proliferation of corneal endothelium and corneal wound healing (Schulz et al., 1996; Klender and Sheardown, 2004). Most uveal melanoma cell lines express FGF subtypes including b-FGF to various extents, and increased FGF expression long with other growth factors was reported in an animal model of retinal detachment (Nakazawa et al., 2006). FGF is also a promising neuroprotective drug against some retinal degenerative diseases and protects retinal photoreceptors from light damage.

An anti-FGF mAb (no registered brand name to date, BioWa, Princeton, NJ, USA) was developed recently for future application treat various cancers. Although no study has reported if that anti-FGF agent is useful in ocular pharmacology, some potential indications for the application of anti-FGF mAb based on FGF function can be proposed as adjuvant chemotherapy for ocular melanoma, in conjunction with other mAbs such as anti-TNF to treat PVR associated with rhegmatogenous retinal detachment, and to reduce the chance of PCO after cataract surgery (Chamberlain and McAvoy, 1987). More investigation should unravel the usefulness of anti-FGF mAbs in PCO or PVR, because so far the absence of a cause–effect relationship has not been settled. In addition, other mediators may play a more important role than FGF in these entities.

6.6. mAb anti-IGF

IGF contains 70 amino acids and may be produced primarily by the liver or in target tissues. Its primary action is mediated by binding to specific IGF receptors on many cell types in various tissues. IGF-1 is a potent stimulator of cell growth/multiplication and a potent inhibitor of programmed cell death in various organs such as bone and the eye, particularly the ocular vascular endothelial cells, RPE cells, and neuronal cells (Sivakumar et al., 2008). Ischemia may stimulate the intraocular production of IGF receptors and thus may be related to angiogenesis. IGF-I also stimulates cell proliferation and migration of RPE cells and to induce RPE cell-derived release of plasminogen activators. The effect of IGF-1 on angiogenic cells in ocular neovascularization may point toward a role for this growth factor in the pathogenesis of neovascular AMD. The proliferative cytokine also may have fundamental roles in proliferative and angiogenic retinal diseases such as AMD, DR, and ROP. For instance, knockout mice for IGF-1 show diminished retinal neovascularization, patients with PDR have increased IGF-1 levels, and restoration of IGF-1 to levels in utero may help prevent ROP (Wilkinson-Berka et al., 2006). αIR3 (no company for therapeutics as of 2008), a mouse anti-IGF-IR antibody, suppresses IGF activity and tumor formation and growth in xenograft models of human breast cancer. Future clinical and experimental research may clarify the benefit of target mAb against human cytokines for treating various ocular diseases. Recommendations for future research of anti-IGF mAbs may point toward use in PDR and ROP, especially in animal models before future consideration in humans. For chorioretinal and corneal neovascularization, further research to elucidate the mitogenic factor is warranted since only low yield data are available.

7. Role of therapeutic mAbs against cluster of differentiation antigens in ocular diseases

Cluster of differentiation antigens are human leukocyte cell-surface markers expressed to distinguish cell lineages, especially

lymphocytes; while in the ocular environment, cluster of differentiation markers may be expressed in ocular disease cells and surrounding tissues. Therapeutic mAbs currently are undergoing clinical investigation, mostly against 20, 11a, 25, and 52 cluster of differentiation antigens (Vitetta and Ghetie, 2006). CD20 is a membrane phosphoprotein expressed on normal and malignant B-lymphocytes, which allows the influx of calcium required for B-cell activation. CD20 is a mediator in oculocerebral or uveal lymphoma, ocular Epstein–Barr virus, uveitis, and even reactive hyperplasia after LASIK (Gündüz et al., 2006). Ibritumomab tiuxetan is the murine IgG1 mAb against CD20 investigated in patients with relapsed/refractory lymphoma, while rituximab (Rituxan®, Genentech, South San Francisco, CA, USA) is a chimeric mAb anti-CD20 antigen that is FDA-approved for subcutaneous infusion in patients with recurrent low-grade B-cell lymphoma; it also may be indicated for RA, systemic lupus erythematosus, leukemia, and Wegener's granulomatosis (Coiffer, 2007; Salvi et al., 2006). Rituximab acts by both complement-dependent and antibody-dependent cell-mediated cytotoxicity and can also induce apoptosis directly. There is growing evidence that new biologic agents such as rituximab may be effective and can be used in patients who do not respond to TNF inhibition using infliximab or adalimumab for systemic inflammation. Such information has clinical impact on the therapy for uveitis; indeed, a small clinical series found rituximab effective for patients with refractory scleritis, orbital inflammation, and intraocular and extraocular lymphoma (Lim et al., 2006). An interesting observation obtained from systemic use of rituximab is that administration of rituximab after autologous bone-marrow transplantation for lymphoma achieved numerous molecular remissions, suggesting that rituximab is even effective in eliminating minimal residual disease. This may imply a greater efficacy and bioavailability of rituximab, which may suggest that high concentrations of the drug may enter the ocular tissues and the novel mAb may promote clinical remission of severe diseases.

The intravitreal pharmacokinetics of rituximab has been investigated recently; the anti-CD20 mAb showed sustained high concentrations with a half-life of 4.7 days in one study, whereas in a second study the agent injected intravitreal in doses up to 1 mg did not induce retinal damage in rabbits or clinical signs of toxicity in a small series of five patients (Kim et al., 2006; Kitzmann et al., 2007; Salvi et al., 2006). Knowledge of the intraocular safety of rituximab may enable intravitreal injection to treat uveal/oculocerebral lymphoma and uveitis. Clinically, anti-CD20 mAbs are beneficial for treating intraocular lymphoma and uveitis, although substantial clinical experience is warranted to clarify the indications and risks of anti-CD mAb. In the near future, rituximab should be compared to mAbs and non-biologic therapies such as corticosteroids for treating ocular inflammatory diseases. Beyond this, rituximab may be tested intravitreally in patients with the rare entity of ocular leukemia.

CD25, also referred to as IL-2 alpha, is a glycoprotein expressed on activated T/B cells and macrophages considered the receptor for IL-2 within lymphocytes expressed in active inflammatory diseases. The private receptor for IL-2, the IL-2R-alpha or CD25, has become a target for immune intervention in the past years. The scientific basis for this approach is that resting normal cells do not express IL-2-alpha, whereas it is expressed by a proportion of the T-cells involved in organ allograft rejection, by T-cell-mediated autoimmune disease, and by specific leukemias and lymphomas. Daclizumab (Zenapax®, Roche, Basel, Switzerland) and basiliximab (Simulect®, Novartis, Basel, Switzerland) are chimeric anti-CD25 mAbs used to prevent rejection in organ transplantation, especially in kidney transplants; it also may be useful as adjuvant therapy for multiple sclerosis, psoriasis, leukemia, or lymphoma. A limitation in the use of unmodified mAbs to treat systemic and ocular leukemia/lymphoma is that they are relatively ineffective as cytocidal agents. Moreover, in ophthalmology the anti-CD25 agents, especially daclizumab, have remarkable potential for treating noninfectious intermediate and posterior uveitis; phase 1 and 2 pilot clinical trials have shown that subcutaneous daclizumab every 2 weeks at 1 mg/kg for 6 months is well tolerated and may reduce the need for concomitant immunosuppression to treat noninfectious uveitis, although the novel mAbs are unsuitable as single drug therapy (Lim et al., 2006). In addition to uveitis, daclizumab could be used to treat other human T-cell-mediated autoimmune disorders. For instance, allograft rejection in corneal transplantation is primarily a T-cell-mediated disease; therefore, ophthalmologists may consider trials with daclizumab for that task. Finally, additional diseases mediated fully or partly by the T-cell immune response with a potential target for daclizumab include chronic allergic conjunctivitis, chronic dry eye disease, or thyroid-associated ophthalmopathy, but this hypothesis must be proven in pre-clinical studies before a final recommendation can be made.

CD52 antigens are expressed on all lymphocytes and may be lytic for target cells, both with human complement and via antibody-dependent cellular cytotoxicity. Alemtuzumab (Campath®, Genzyme, Cambridge, MA, USA) is a humanized mAb against the CD52 antigen developed to treat myeloma, and leukemia and systemic inflammation secondary to Behcet's disease. Alemtuzumab decreased inflammation in a series of patients with severe refractory ocular inflammatory diseases associated with various systemic diseases such as RA or Behcet's disease, but no clinical trial has been registered at www.clinicaltrials.gov (Dick et al., 2000). In addition, single case studies have reported the success of Campath-1H therapy for controlling refractory severe posterior uveitis, peripheral ulcerative keratitis associated with Wegener's granulomatosis, and recurrent corneal graft rejection (Wertheim et al., 2006). Campath-1H may enable restoration of ocular immunoregulation by altering lymphocyte subset ratios, but the problem is that both autoaggressive and regulatory T-cells reside within the CD4+ subset cells. This type of problem can be illustrated by a recent report on the development of cytomegalovirus retinitis after allogeneic hematopoietic stem cell transplantation using an alemtuzumab-based conditioning regimen (Song et al., in press). In addition, alemtuzumab may promote a high incidence of infusion reactions and considerable hematologic toxicity and infusion reactions, which may explain why this form of mAb has not achieved the same widespread popularity of the TNF inhibitors. Although alemtuzumab may not be proposed as a first-line agent for ocular inflammation, it should be considered as adjuvant therapy in cases of severe refractory ocular inflammation and to prevent or as therapy for rejection after corneal transplantation. In addition, the efficacy of combined alemtuzumab with rituximab for ocular immunologic diseases should be investigated.

CD11a/CD18 is involved in leukocyte adhesion during cellular interactions essential for immunologic responses on antigen-presenting cells, endothelial cells, and keratinocytes. The humanized anti-CD11a mAb efalizumab (Raptiva®, Genentech, South San Francisco, CA, USA) is highly effective for bone-marrow transplantation and psoriasis. Efalizumab binds to the α-subunit of CD11a of leukocyte-function-associated antigen 1 (LFA1), a member of the integrin family of cell-adhesion molecules that is expressed on all leukocytes. It thereby blocks interaction between LFA1 and intercellular adhesion molecule 1 (ICAM1), a molecule in the initiation and maintenance of multiple processes involved in the pathogenesis of inflammatory diseases such as psoriasis, including T-cell activation and migration of T-cells into the skin. In the ocular environment CD11a may be found in corneal wound healing, DR, PVR, and uveitis (Li et al., 2006). Evidence to date

showed that an earlier produced anti-CD11a mAb inhibited development and clinical inflammation in the animal model of EIU (Whitcup et al., 1995). These preliminary data indicate that anti-CD mAbs are a plausible approach to treat inflammatory ocular diseases, abnormal corneal wound healing, or PVR, but substantial data on the cause–effect relationship of CD11a in the various ocular pathologies are necessary.

8. Role of therapeutic mAbs against complement proteins in ocular diseases

The complement system is an inflammatory cascade that assists in the clearing pathogens and modulation of immunologic reactions. Over 20 proteins and fragments make up the complement system. One, C5, is catalyzed into derivate components to ultimately induce formation of the membrane attack complex. AMD is a multifactorial disease that may involve perfusion, tissue aging, and photochemical, oxidative, and inflammatory factors. Of note, the inflammatory response in AMD has members of the complement cascade such as C3 or C5 that induce pathologic changes such as drusen or CNV.

Eculizumab (Soliris®, Alexion Pharmaceuticals, Cheshire, CT, USA), a humanized mAb directed against the complement protein C5, was FDA-approved on March 16, 2007, for treating paroxysmal nocturnal hemoglobinuria. The manufacturer has started pre-clinical studies to evaluate the role of intravitreal injections of eculizumab for wet AMD. Anti-complement mAbs may have a substantial clinical impact in the future, because they also may act on dry AMD, since the number of patients with this type of AMD is far greater than the patients with wet AMD.

9. Future directions and conclusions

mAbs entered clinical practice in the early 1980s, but the lack of efficacy and rapid clearance secondary to human anti-mouse antibodies became apparent. Chimeric mAbs are partly clinical superior to humanized mAbs due to the difficulty of producing large amounts. However, advances in genetic engineering facilitate production of humanized and human mAbs and their fragments. mAbs currently play a central role in cancer immunotherapy and inflammatory and rheumatologic diseases; about one-third of pharmacologic agents available in clinical practice are mAbs. While inflammatory diseases mAbs inhibit the activity of proinflammatory molecules and cytokines, in cancer antibodies exert anti-tumor effects by inducing apoptosis, preventing the expression of proteins critical to the neoplastic phenotype or disturbing vital structures such as the tumor vasculature. The number of mAbs entering pre-clinical and clinical research has increased. Among the FDA-approved mAbs, the chimeric and humanized mAbs comprise 14 of the 17 approved mAbs. While three are murine products, one human-mAb adalimumab was approved by the FDA in 2002.

The power of mAbs to unravel the pathophysiology of complex multifactorial immune-mediated inflammatory diseases is demonstrated by infliximab, the anti-TNF-α mAb, that has outstanding efficacy in systemic inflammatory diseases. This feature is particularly notable because it reveals the positive outcome of a drug that blocks a single cytokine in the diverse bouillabaisse of inflammatory mediators associated with many human diseases. The marked efficacy of anti-TNF-α mAbs in Crohn's disease and RA indicates that even in immune disorders in which many mediators are implicated, a small number might be markedly superior. In turn, the efficacy of anti-TNF mAbs for various human diseases as RA, psoriasis, and Crohn's disease indicates that those entities may share many molecular and cellular pathophysiologic characteristics. Overall, the therapeutic advantages of mAbs over conventional small-molecule drugs are many, i.e., high target specificity and drug organization into distinct structural domains, which enables changes in affinity, avidity, bio-distribution, and half-life.

However, the mAbs currently available are proteins and, therefore, may have poor stability, poor cellular penetration/activity, resistance, little oral absorption, a short half-life, variable immunogenicity, and rapid metabolism. To overcome these problems, future trends in biologic therapy include the greater importance of mAb fragments, human mAbs, and clinical studies for mAbs directed against other molecular factors, cytokines, or accessory molecules. In addition, novel construction methods may lead to a generation of small molecular anti-cytokine agents, called superantibodies, to target specific signaling and/or biosynthetic pathways. Superantibodies may enhance mAb potency, promote better cell membrane penetration, or express bifunctional antibody activities. Another approach for enhancing the effector functions of antibodies is the engineering of bispecific antibodies, which comprise two specificities, one for the cell to be eliminated and one for receptors on effector cells such as cytotoxic T-cells. Moreover, another approach to for increasing the clinical efficacy of antibodies lies in attachment of antibodies to liposomes containing drugs, anticancer drugs, radioisotopes (radioactive substances), or toxins (immunoconjugates) to deliver substances like poisons directly to the tumor, thereby helping to destroy it. Two radioimmunoconjugates, ibritumomab tiuxetan and tositumomab-131I, and one drug, conjugated gemtuzumab ozogamicin, are available for oncology; the therapies have shown considerable discrimination between target and normal tissue, thereby providing better efficacy and less toxicity. There is an unexplored area of mAbs research in ophthalmology, although no specific immunoconjugate has been proposed so far. Further specific tasks of ongoing and future studies should include an understanding of the mechanisms of resistance, identifying predictive markers of response or resistance, and establishing reliable assays for clinical use.

Some additional disadvantages of mAb application are high cost; infliximab therapy costs approximately US$20,000 annually per patient, depending on the circumstances and the country. Therapy for CNV with ranibizumab costs around US$ 30,000 annually. This may be explained by the high expenses of mAb production; development of one humanized mAb is about US$1 billion. However, data from manufacturers suggest that TNF inhibition is cost-effective according to the commonly applied threshold of $50,000 per quality-adjusted life-year gained. The cost of mAb therapy could limit its widespread use because more cost-effective therapies are available.

The anti-TNF mAbs infliximab, etanercept, and adalimumab are effective therapeutic options for refractory intraocular inflammation, with strong basic science research and clinical studies supporting the use of TNF antagonists especially for treating uveitis. The 5 mg/kg dose of infliximab is effective and has been used in most previous studies of uveitis after conventional immunosuppressive drugs failed; this approach may obtain a sustained response over the long term if infusions are administered every 8 weeks. Studies of various types of uveitis indicate that Behcet's disease- and juvenile idiopathic arthritis-associated uveitis are some of the best indications for the use of infliximab, although the drug may be effective for other types of uveitis. Nevertheless, anti-TNF agents do not produce remission of inflammatory diseases. Further important issues concerning anti-TNF therapy that should be investigated in large controlled clinical studies in ocular inflammation are the optimal dose/rhythm/and duration of anti-TNF administration; comparison of the relative efficacy of each anti-TNF agent; clarification of anti-TNF mAbs therapy for ocular inflammation compared to

immunosuppressive agents such as corticosteroids; the clinical response of intravitreal anti-TNF mAbs for uveitis, since trials on the efficacy of intravitreal injection of infliximab could be an alternative to systemic administration in selected patients; the cause of the complete failure of anti-TNF mAbs in a percentage of patients with uveitis; and investigation of the role of anti-TNF agents in reducing ocular neovascularization.

The application of anti-VEGF in ophthalmology for intraocular neovascularization has set new standards in terms of improvements in signs of disease and quality of life. Bevacizumab and ranibizumab may undoubtedly induce clinical regression of ocular neovascularization. Ranibizumab received FDA approval for treating wet AMD, whereas bevacizumab also has great efficacy, although its use remains an off-label procedure. It is possible to infer from these studies that monthly injections of ranibizumab are the most effective for treating wet AMD. However, three consecutive monthly injections followed by rigorous evaluations and new injections based on decreased VA, presence of new-onset hemorrhage, increased retinal thickness, and persistent subretinal fluid might be as effective. This offers a new perspective on the appropriate treatment at a lower cost. Importantly, the best time to intervene with an antiangiogenic agent may be earlier in the course of neovascular disease. Future challenges in the research of VEGF antibodies will lie in the clarification of these important issues: selection of patients who are most likely to respond—reliable markers that can predict which patients are more likely to respond to anti-VEGF therapy are important, but so far they have been elusive; determining the doses of mAbs and fragment and scheduling of intravitreal injections; determining the optimal combination of antibodies with other biologic agents, corticosteroids, and immunosuppressive agents; determining the efficacy of anti-VEGF agents for each antineovascular disease; determining the difference in efficacy between bevacizumab and ranibizumab for ocular neovascularization resulting from CNV due to AMD; and studying the causes of failure and resistance to anti-VEGF therapy in ocular angiogenesis.

Future directions for the use of mAbs include adding novel agents targeting other cytokines or growth factors as sole therapy or in combination with anti-VEGF or anti-TNF agents, and the evaluation of proof-of-principle combination regimens in appropriate animal models. Inflammatory and angiogenic pathways become more numerous and redundant as diseases progress. Considering this, it is unlikely that inhibiting one factor or pathway will produce a sustained clinical effect in patients with previously treated, highly refractory disease. In initial experimental and clinical experience, rituximab may be a promising approach for treating ocular lymphoma and leukemia, while daclizumab and alemtuzumab may be indicated as therapeutic options for uveitis or other primarily T-cell-mediated diseases such as corneal rejection after transplantation. Efalizumab may have potential as adjuvant agent for any ocular inflammatory disease in which leukocyte adhesion is important, including uveitis, and eculizumab has great potential for dry and wet AMD. Although endogenous mAb administration has acceptable toxicity profiles, intraocular injection may minimize systemic complications and increase the drug amount available to intraocular tissues such as the retina. However, mAbs may inhibit physiologic molecular components of ocular and non-ocular tissues, and the long-term side effects of such inhibition are unclear. Therefore, additional long-term data are required to determine the relative benefits and drawbacks of the use of different mAbs in ocular diseases. The introduction of biologic therapeutics with mAbs may facilitate unmet clinical needs in many ophthalmic diseases. We have entered an exciting era of biologic agents that is just the beginning of a revolution in therapeutics that is giving hope to many patients.

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