1991

Do calcuim channel blockers protect against first dose reaction to OKT3?

Eric A. Richard
Yale University

DO CALCIUM CHANNEL BLOCKERS PROTECT AGAINST FIRST DOSE REACTION TO OKT3?

Eric A. Richard

1991

Permission for photocoping or microfilming of "Do calcium channel blockers protect against first dose reaction to OKT$_3$"

(Title of thesis)

for the purpose of individual scholarly consultation or reference is hereby granted by the author. This permission is not to be interpreted as affecting publication of this work or otherwise placing it in the public domain, and the author reserves all rights of ownership guaranteed under common law protection of unpublished manuscripts.

Erica Richard

Signature of Author

3/15/91

Date

Do Calcium Channel Blockers Protect Against First Dose Reaction to OKT3?

A Thesis Submitted to the Yale University School of Medicine in Partial Fulfillment of the Requirements for the Degree of Doctor of Medicine

by
Eric A. Richard
1991

DO CALCIUM CHANNEL BLOCKERS PROTECT AGAINST FIRST DOSE REACTION TO OKT3? Eric A. Richard, and Margaret J. Bia. Section of Nephrology, Department of Internal Medicine, Yale University School of Medicine, New Haven, CT.

Abstract

OKT3 is a monoclonal antibody used as an immunosuppressant to treat rejection or to prevent cyclosporine toxicity in the setting of post-operative delayed graft functioning. Use of OKT3 is often complicated by a first dose reaction, ranging from a mild reaction to a life threatening complication. OKT3 binding to human T lymphocytes promotes an influx of calcium, which stimulates mitogenesis and a release of lymphokines (the proposed cause of this clinical syndrome). Since calcium channel blockers can inhibit T lymphocyte activation in vitro, this study was performed to determine if clinically used doses of calcium channel blockers decrease the severity of first dose reaction to OKT3.

A retrospective, chart review study was employed to analyze the incidence of side effects of patients on calcium channel blockers (N=20) as compared to those who were not (N=49). The calcium channel blocker group consisted of 13 patients on nifedipine (30-120 mg/day), 5 on verapamil (120-480 mg/day), and 2 on diltiazem (90-180 mg/day). All patients received OKT3 (5mg) after being premedicated with acetaminophen, diphenhydramine, and varying amounts of glucocorticoids. The two groups were similar in age, sex, race, percent on dialysis, and percent who had received

cadaveric transplants. Dose of azathioprine, dose of methyl-prednisolone, and both the dose and level of cyclosporine were also similar in the two groups.

There were no differences between the two groups in the frequency of the fever or the magnitude of the fever spike. The two groups were also similar in the frequencies of chills, GI upset, hemodynamic changes, and respiratory symptoms. Only the symptom of fatigue was different, occurring more often in the calcium channel blocker group. The data suggest that clinically used doses of calcium channel blockers do not protect against first dose reaction to OKT3 perhaps because these doses do not achieve plasma levels high enough to inhibit T cell activation and subsequent lymphokine release.

Acknowledgements

I am grateful to Dr. Margaret Bia for all her assistance. Her wisdom, encouragement, and enthusiasm made this project both educational and enjoyable. I thank Dr. Marc Lorber for his help in accessing his transplantation files and for his critique of this paper. I thank Dr. Joanne Elmore for her insightful comments regarding clinical projects and for her assistance with the statistical analysis. I thank Dr. James Jekel for his review of the statistical methods. Finally, I thank my family and my wife for their support throughout this project and all of medical school.

Introduction

The monoclonal antibody OKT3 is a powerful immunosuppressant used in renal transplant patients as therapy to treat resistant rejection and to avoid cyclosporine nephrotoxicity in the clinical setting of post-operative delayed graft functioning. First and second doses of OKT3 are associated with a well described clinical syndrome that is known as "first dose reaction to OKT3". The syndrome consists of fever, chills, hemodynamic changes, GI upset, and neurological changes. Initial premedication with acetaminophen, diphenhydramine and glucocorticoids has not been able to eliminate this syndrome. Although infrequently life threatening, this adverse drug reaction is an important source of patient discomfort. This adverse reaction may result from an OKT3 induced T cell activation, leading to the production and release of lymphokines. This activation appears to use calcium as a second messenger, and, in vitro, calcium channel blockers decrease the production of lymphokines. This study examines whether clinically used doses of calcium channel blockers can decrease the first dose reaction to OKT3.

General Description

Human T cells detect antigen, leading to subsequent activation and proliferation, via a T cell receptor(TCR)/CD3 complex (1). Proof (2) that a TCR/CD3 complex exists includes that CD3 immunoprecipitates with TCR, CD3 comodulates with TCR, and a stoichiometric relationship exists between CD3 and TCR. Consisting of α and β subunits, the TCR recognizes the specific antigen; the CD3

subunit, which is non-covalently linked to the TCR ß subunit, transduces the antigen-binding signal into the T cell. Phosphorylation of the CD3 complex post TCR occupancy (3) lends proof that CD3 is the signal transducer. Furthermore, human monoclonal antibodies which bind to the CD3 complex induce an increase in intracellular calcium (2,4), T cell proliferation (2), and lymphokine production (5,6,7,8) - all similar events to what occurs after antigen binds to the TCR. Present on all mature T cells and late thymocytes (1), the CD3 complex consists of 3 invariant subunits - 2 glycoproteins (γ and δ) and 1 non-glycosylated hydrophobic polypeptide (ε). Because its intracellular component is larger than its extracellular, CD3δ is postulated to be the actual signal transducer in man.

Initially developed in 1978 to differentiate between T cell subsets (9), the monoclonal antibody OKT3 binds to a 20kd subunit of the CD3 complex. In fact, the term CD3 was subsequently derived from OKT3. The first monoclonal antibody used in humans, OKT3 is an IgG2a immunoglobulin and consists of a 50kd heavy chain and a 25kd light chain. Derived from a hybridoma and grown as an ascites in pathogen free, standard bred mice, OKT3 is purified to be free of both pyrogens and pathogens.

Immunosuppression

OKT3 exerts its powerful immunosuppressive actions (10,11,12,13) by removing CD3+ cells from the circulation (usually within 1 hour) and by blocking the killer function of sessile T cells (the would be killer cells in the allograft) - as described below.

After the OKT3 antibody binds to the CD3 complex, this complex is opsonized and is either removed by the reticulo-endothelial system via lysis or redistributed to lymph nodes. Furthermore, similar to high levels of antigen, OKT3 effectively modulates, or removes, not only the CD3 antigen from the T cell surface but also the TCR, making these T cells non-functional.

Studies by Gebel(14) have shown that in renal transplant patients, circulating CD3 cells are diminished from 70% to <1% after one dose of OKT3. Within 2 days, CD3 cells returned to the circulation with greatly decreased density of the CD3 molecule and no co-expression of TCR. Failing to proliferate post allogenic stimulation in vitro, these CD3- cells were nonfunctional and immuno-incompetent. Because CD3+ cells returned to the circulation and lymph nodes once OKT3 was stopped, this process was reversible. Similarly, Zlabiger(15) and Caillot-Zucman(16), by showing modulation of CD3 cells, added further evidence for this phenomenon. As T cell depletion was only 20-60% in the lymph nodes, modulation plays a more vital role. More importantly(17), in the allograft, some CD3+ cells were present but nonfunctional and without the TCR. Therefore, because of the effects of modulation, the modulated T cells were incapable of mounting an immune response against the allograft. Reappearance of the CD3 complex may have indicated failure of therapy, perhaps secondary to the development of anti-OKT3 antibodies.

The inhibition of T cell killing capacity is another powerful immunosuppressive mechanism of OKT3. Landegren(18), using cell

cultures of peripheral blood lymphocytes from healthy volunteers, showed that OKT3 blocked cytotoxic T cell lysis by impeding the ability of the cytotoxic T lymphocytes to lyse bound cells, rather than by reducing target binding. However, Seventer(19), using a panel of anti-CD3 monoclonal antibodies which included OKT3, demonstrated that OKT3 inhibited target cell recognition by the cytotoxic T lymphocytes via steric hindrance or confirmational changes. Nonetheless, the cytotoxic T lymphocytes blocking capacity occurred in vitro at normal therapeutic trough levels of 1ug/ml (for 5mg dose).

Clinical Trials

Many clinical trials have suggested that OKT3 is successful when used as treatment against rejection (both primary and resistant) and as prophylaxis in the early post transplant period. Treating 8 cadaver renal allograft recipients undergoing acute rejection, Cosimi(20) reported on the first clinical trial using OKT3. This monoclonal antibody offered several advantages over polyclonal antibodies - mainly homogeneity, ease of use and monitoring, and lower toxicity. Within 2-7 days, OKT3 reversed rejection in all 8 case with a 75% 12 month graft survival. Similarly, in a large, prospective, randomized, multicenter study(21), 123 patients were treated with 14 days of OKT3 vs conventional high dose steroids. The results showed reversal in 94% vs 75% and 1 year survival at 62% vs 45% (OKT3 vs steroids). The efficacy of OKT3 in these initial trials set the stage for use in other transplant settings.

Many studies have suggested that OKT3 is efficacious in the primary treatment of rejection (22,23) and in the treatment of both steroid resistant (23,24,26-30) and ATG/steroid resistant rejection (22,24,30). Some investigators(28) suggested treating rejection with a pulse of high dose steroid; if successful, this obviates the need for OKT3 therapy. Some have also shown some success with OKT3 as a prophylactic agent against rejection (25,34-40) and in the setting of post-operative delayed graft functioning (25,31), where clinicians are hesitant to use cyclosporine with its associated nephrotoxicity. Researchers (32,33) have used OKT3 concomitantly with cyclosporine with good success in the treatment of rejection; in fact, cyclosporine may have reduced the formation of anti-OKT3 antibodies and increased the efficacy of OKT3 (32).

If it has been used as a prophylactic agent against rejection in the early post-op period, OKT3 can be re-used to treat rejection episodes (41,42). Because OKT3 is a murine derived product, antibodies to OKT3 can form after initial administration of OKT3. Presence of low titre anti-OKT3 antibodies does not preclude re-treatment with OKT3. However, the number of doses of OKT3 needed to deplete CD3 cells from the circulation can be greater in the retreated patients as compared with first time receivers of OKT3 (41). Furthermore, it may be necessary to increase the dose of OKT3 in retreatment groups to achieve adequate serum OKT3 levels and to deplete CD3 cells. Presence of high titre antibodies is a contra-indication to re-use of OKT3(41) as OKT3 is rarely effective in this situation.

Side Effects of OKT3

In the first clinical trial (20), which used a small patient sample of 8 patients, chills, a febrile response and an occasional wheeze on the first day were noted. This adverse drug reaction appeared quite mild and was easily treated with antihistamines and acetaminophen. In the multicenter study (21) in 1985, adverse drug reactions were first recognized as being important. These reactions began 45-60 minutes after the first injection (sometimes second, and never subsequent) and lasted several hours. Reported signs and symptoms included fever (73%), chills (57%), tremor (10%), dyspnea (21%), chest pain/tightness (14%), wheeze (11%), nausea (11%), and vomiting (13%). One patient in a state of fluid overload developed pulmonary edema. Because of the timing of these signs and symptoms after the first or second dose (and rarely subsequent), the syndrome has been labeled "first dose reaction to OKT3".

Subsequent studies (20,22,25,27,34,38,43,44) have shown similar side effects, in addition to others, occurring at significant rates. The signs and symptoms that have occurred frequently enough so that multiple investigators have reported them include fever, chills, hemodynamic sequelae (tachycardia, changes in blood pressure), gastro-intestinal upset (vomiting, diarrhea), CNS changes (headache, seizure, malaise, aseptic meningitis), respiratory distress (dyspnea, wheeze, chest pain/tightness) and arthralgias/myalgias. The symptoms of acute rejection can mimic OKT3-related symptomatology. Similarly, OKT3 reactions are difficult to distinguish from normal post-op recovery.

Fever and chills, respiratory and hemodynamic sequelae are the most frequent first dose side effects which occur in the first few hours (43). Hypertension and mild dyspnea are associated with a state of relative fluid overload. This is why many protocols include a diuresis/dialysis to <103% of ideal body weight prior to OKT3. Furthermore, hypotension and tachycardia have been associated with a state of relative volume depletion, often secondary to over aggressive diuresis and dialysis.

Gastro-intestinal and CNS side effects usually appear between days 2-3 of OKT3 therapy. These symptoms are usually transient and self-limiting and stop after OKT3 is withdrawn.. Aseptic meningitis with symptoms of headache, fever, photophobia, nuchal rigidity, and mental status changes (43) has occurred in a small percentage of patients. Lumbar puncture shows leukocytosis, elevated protein, normal glucose, and negative cultures. One possible cause (45) for aseptic meningitis is that OKT3 cross reacts with a neural antigen, promoting local inflammation. Seizures can also occur, with one study (43) showing an association with post-op delayed graft functioning.

Mechanism of Reaction to OKT3

From the initial studies, it was suggested that the first dose reaction was caused by a release of endogenous pyrogens from lysed T cells (21). These first dose symptoms were not attributed to hypersensitivity for the following reasons - 1) they occurred in almost all patients without previous exposure to murine immunoglobulin, 2) skin tests prior to OKT3 were routinely negative,

and 3) symptoms did not recur with later injections. Because many of the side effects were similar to those after systemic administration of interleukin-2 (IL-2) and after endotoxic shock mediated by tumor necrosis factor-α (TNF-α), lymphokines began to be investigated as the cause of the OKT3 induced clinical syndrome.

Lymphokines are well characterized as mediators of both inflammatory and immune reactions (12,46,47,48). Interleukin-2 (IL-2), produced in T cells and known also as T-cell growth factor, has been clearly implicated in inducing T cell proliferation and differentiation. Rosenberg (46) described an adverse clinical reaction in cancer patients who received high dose IL-2 with lymphokine activated cells. Symptoms reminiscent of OKT3 included fever, vomiting, diarrhea, fatigue, and chills. Furthermore, he described a scenario of increased capillary permeability (leading to pulmonary edema) and decreased vascular resistance (leading to hypotension). In fact, 34 out of 180 treatment courses were complicated by pulmonary edema; 16 patients required intubation (46).

Tumor necrosis factor-α (TNF-α), or cachectin, has been implicated as a mediator of cachexia in cancer patients and endotoxic shock in patients with gram negative sepsis. Produced by both monocytes and T cells, TNF-α causes piloerection, diarrhea and withdrawal in mice, hypotension, tachypnea and respiratory arrest in rats, and fever in rabbits. Remick (47) studied the in vivo effects of human recombinant TNF-α in mice and induced hypovolemic shock, necrosis of the small bowel, fever, hypotension, malaise, and

respiratory failure. Kinkhabwala (49) has shown that normal activated T cells express TNF-α on their cell surface. He postulated that cell bound TNF, which is an important mediator of the inflammatory response and is implicated in host defense, is advantageous while the secreted TNF mediates the explosive clinical syndrome. Likewise, interferon-γ (IFN-γ), produced by T cells, seems to work in synergism with TNF for cell cytotoxicity.

OKT3 and Lymphokine Production

Because OKT3's first dose reaction was similar to lymphokine induced clinical symptoms, investigators attempted to measure if lymphokine levels were increased after OKT3 administration. Suthanthiran (5) demonstrated that OKT3 added to peripheral blood lymphocyte culture promoted an increase in the level of IL-2 and IFN-γ.

Abramowicz (8) studied renal transplant recipients who received OKT3 for nonrejection prophylaxis. Lymphokine release was measured after exposure to OKT3 (with 1mg/kg of methyl prednisolone) or to cyclosporine at 6 mg/kg. In the cyclosporine patients, TNF did not increase, and IL-2 and IFN levels were undetectable. In contrast, OKT3 induced a marked rise in IL-2 (peak 2 hr), IFN (peak 2 hr) and TNF (peak 1 hr). Lasting only 24 hours, these rises were transient. Furthermore, in 78% of the OKT3 treated patients, subsequent doses of OKT3 did not induce a significant release of these lymphokines.

Meanwhile, Chatenaud (7), using 17 patients, showed that the first, and only the first, dose of OKT3 caused a sharp increase of

IFN-γ and TNF-α. In 3 patients who had received a haploidentical graft from a family member and had not received steroids, IL-2 was also increased. The peaks of lymphokine levels were similar to the previous study except that IFN and IL-2 both peaked at 4 hours. The rise in lymphokine levels resolved in 15-20 hours and did not recur with the second dose. No changes in IL-1ß and IFN-α were detected at any time point. In an extension of the study to 35 patients, similar results were obtained (6). This increase in lymphokines paralleled temporally the reversible clinical syndrome. The difference in the two studies (6 and 8) which may explain the different IL-2 levels is that Abramowicz used much lower doses of steroids than Chatenoud (1mg/kg vs 1g).

Bloemena (50) has recently implicated IL-6, an inducer of acute phase hepatic proteins, as another mediator. He suggested that even though IL-6 levels are raised during rejection, they are increased significantly more after OKT3. Because the peak of IL-6 was 48 hours, he suggested that IL-6 could mediate some of the late side effects associated with OKT3.

Multiple studies (50-54) have shown that all lymphokines are elevated during rejection. However, the level of circulating lymphokines increases even more after OKT3 administration.

OKT3-Tcell activation-Lymphokines

Thus far, the ideas that OKT3 causes a first dose reaction, that this reaction mimics the clinical picture of lymphokine release, and that OKT3 causes an increase in lymphokine release (in vitro and in vivo) have been discussed. Other studies have indicated that T cells

are responsible for lymphokine production after activation by other mitogens (55-58). How does one resolve the conflicting evidence that OKT3 is a powerful immuno-suppressant and that OKT3 induces a release of lymphokines, products of T cell activation? Prior to examining blood levels of lymphokines, most investigators (10,20,21,59,60,61,62) explained the adverse drug reaction to OKT3 as resulting from endogenous pyrogens which were released by opsonized cells, lysed in the reticulo-endothelial system. Some problems with this theory include that other anti-T cell antibodies which induce opsonization do not cause a similar syndrome and that very small amounts of active lymphokines are stored within lymphocytes.

Therefore, another mechanism was needed to explain how OKT3 induced a lymphokine increase - OKT3 is not only a powerful immunosuppressant but also a T cell mitogen, capable of inducing activation and proliferation. In 1980, van Wauwe (63) showed that OKT3 was a potent mitogen of peripheral lymphocytes in vitro. Measuring 3H thymidine incorporation as an indication of T cell activation, he showed that OKT3 had definite mitogenic activity, even greater than phytohemagglutinin (PHA) and concanavalin A (CON A). Furthermore, OKT3 displayed no inhibition of response at high concentrations.

Von Wussow (64) demonstrated that T lymphocytes which expressed CD3 antigen produced IFN after OKT3 was added to the medium. The timing of maximum activity of IFN (3-6 hours) correlated with in vivo studies (4 hours). Chang demonstrated that a

certain subset of T cells, CD4+ cells, secreted IFN (peak 3 hours) and that macrophages played an accessory role. Another study (65) implied that OKT3 induced IL-2 and IL-2R production, while others (66) showed that OKT3 induced production of TNF mRNA. Furthermore, Suthanthiran (5,13), performing in vitro studies, demonstrated that OKT3 caused T cells to become activated, induced both secondary cytolytic activity and natural killer activity, and increased the levels of IL-2 and IFN.

Because most circulating blood cells are CD3- after OKT3 use, Ellenhorn (67) examined lymph node cells and offered the first in vivo support for T cell activation by OKT3. OKT3 coated lymph node T cells showed increased proliferation in vitro in presence of IL-2 and increased expression of IL-2R. Several studies (4,68,69,70) have demonstrated a requirement of macrophages for OKT3 induced activation to occur. Ceuppens (70) discussed a family which failed to respond mitogenically to OKT3 but did respond to other mitogens; this lack of response was restored with the addition of macrophages. In contrast, others have demonstrated mitogenesis in macrophage free media (5,63,64,66).

The clinical setting of OKT3 administration may play an important role in the amount of lymphokines released and the severity of the subsequent first dose reaction. When used in the acute post-operative period, OKT3 may have different side effects than when used for treatment of rejection - although some investigators found no difference(37). Postulating that T cells had not been activated by rejection, some researchers(34,39,71) implied

that OKT3 used as prophylactic agent had fewer side effects. Also, the drug was often-times giving peri-operatively, and the patient, under anesthesia, would have been unable to report some of the side effects. Others (25) believed that prophylactic use of OKT3 carried increased risk for adverse drug reactions because there was no prior exposure to immunosuppressants.

Concomitant immunosuppression plays an important role in cytokine release from T cells and in OKT3 related symptoms. Cyclosporine may act in vitro to inhibit mitogen directed activation(72), permit suppressor T cell proliferation(73), and inhibit production of both IL-2 (74) and IFN (75). Also, corticosteroids(51,75) inhibit IL-1 dependent release of IL-2, inhibit IFN release and have general lymphopenic effects (56,76,77) - which would decrease the number of T cells exposed to OKT3.

Several investigators have examined if concomitant immunosuppressives affect lymphokine levels post OKT3 administration. Suthanthiran (5) has shown that cyclosporine and methyl prednisolone caused a marked inhibition of memory T cell proliferation and of cytotoxic lymphocyte activity. IL-2 production was markedly decreased by cyclosporine and mildly by methyl prednisolone; both equally inhibited release of TNF. Another investigator (6) determined that methyl prednisolone exerted its greatest effects in decreasing OKT3 related side effects if it was given in high doses (500mg) 15-60 minutes prior to OKT3.

Gaston published a report (78) indicating the importance of TNF as the cause of OKT3 related side effects. He used a graded

scale (0-3) for four symptoms (fever, headache, dyspnea, rigors). Patients were separated into two groups, based on the number of side effects reported. The total number of CD3+ cells and of CD4+ cells prior to OKT3 administration were higher in the group with the more severe reaction. As with other studies, the peak of TNF at 2 hours correlated well with the severity of the reaction. He showed no rise in IFN or IL-2. All patients were premedicated with 250 mg of methyl prednisolone which could account for the lack of increase in IL-2. He postulated that T cell activation rather than lysis was the cause of the increased levels of TNF and the subsequent adverse reaction.

Based on the above mentioned studies, it is now thought that OKT3 is a powerful immunosuppressant and it possesses immuno-activating abilities that are dose related. Because CD3+ cells are removed from circulation (by opsonization or modulation) and are unable to react, lymphocyte activation, and hence clinical reaction to OKT3, may not exist after the first or second dose of OKT3.

Murine Model

A murine model for OKT3 exists which further supports the role of OKT3 as a mitogen, capable of stimulating lymphokine release and producing a symptom complex. Immunizing Armenian hamsters with murine cytolytic T cell clone, Leo (79) has developed a monoclonal antibody (145-2c11) which is a murine analog to OKT3. This monoclonal antibody is directed at the CD3ε component of the TCR/CD3 complex. It can act also as a T cell mitogen, inducing non-antigen specific lysis and T cell proliferation. Hirsch (80,81)

classified further 145-2c11 actions, finding that it produced a rapid depletion of peripheral T cells and a delayed and incomplete depletion of T cells in lymphoid tissue. This antibody induced a modulation of TCR and a mitogenic response in T cells. Immunosuppressive abilities of 145-2c11 were demonstrated by increased graft survival time and decreased cytotoxic T lymphocytes activity. Because rapid and extensive lysis did not occur within the first 2 days of therapy, the adverse drug reaction secondary to the monoclonal antibody was probably not due to lysis. They postulated that this reaction may be secondary to the reactivation of latent viruses as the adverse drug reactions did not appear in hepatitis free mice. This monoclonal antibody also caused IL-2R expression, IL-2 secretion, and extra-medullary hematopoiesis in the spleen.

Others (82-85) utilized this monoclonal antibody to address further the issue of OKT3 promoting lymphokine release, a proposed cause of the first dose reaction. In vivo injection of 145-2c11 in mice caused a transient increase in TNF, IFN, IL-2, IL-3, and IL-6 that paralleled a symptom complex consisting of hypothermia, diarrhea, hypomotility, piloerection, and somnolence. Histopathology included cell necrosis and edema in lymphoid organs and edema/congestion of lung, liver and GI tract. Serving as a control, anti-CD4 antibodies did not elicit a lymphokine release or the clinical picture. These studies helped link the clinical reaction to the release of lymphokines because the kinetics of increased lymphokines were superimposable with the clinical picture, the

kinetics were similar to OKT3, and the reaction was similar to that described after lymphokine administration.

Prevention of First Dose Reaction

In an attempt to prevent the first dose reaction to OKT3, various protocols have been implemented. Many protocols have included pretreatment 30 minutes prior to OKT3 with diphenhydramine, acetaminophen, and glucocorticoids. Some protocols also require glucocorticoids (100 mg hydrocortisone) 30 minutes after OKT3 use. However, no study has analyzed the potential protective effects of the different protocols on the incidence or severity of OKT3 induced side effects. One would anticipate that drugs which inhibit lymphokine release after lymphocyte activation might be useful in blocking this reaction.

Calcium Entry - A Step in T Cell Activation

One might postulate that if more were known about the mechanism of OKT3 induced mitogenicity, one could develop a treatment to inhibit the side effects. Tsien (86) helped introduce the potential role of calcium as an important mediator of T cell activation. He developed a technique (Quin 2) to measure directly the intracellular calcium concentration in mitogen stimulated T cells, which showed a 2 fold increase in calcium. Calcium free medium and agents that increased cAMP, which were both known to inhibit mitogenesis, inhibited this calcium response.

In a review of the role of calcium in lymphocyte proliferation (87), Lichtman discussed that calcium uptake by the cell occurred after mitogen stimulation, which lead to 3H thymidine uptake in the

DNA and subsequent mitosis. This activation effect was blocked by EGTA, a calcium chelator. Furthermore, the calcium ionophore A23187 induced both calcium and 3H thymidine uptake and served as a T lymphocyte mitogen. Mills (88,89), also using Quin 2, showed an association between phytohemagglutinin (PHA) induced calcium uptake by cell and 3H thymidine uptake by DNA; both were blocked by EGTA, as was PHA induced IL-2 production. He concluded that T cell mitogenesis did not occur without an initial calcium flux.

Mitogens such as PHA and concanavalin A (con A) may require a calcium flux for T cell activation, but does OKT3? Researchers(2) have suggested that OKT3 promoted a significant increase in intracellular calcium. Using a CD3 negative (CD3-) culture, they also demonstrated that the calcium ionophore could obviate the need for the CD3 antigen in producing mitosis. Because normal mitogens (PHA) did not cause an increase in intracellular calcium in CD3- cells, the researchers linked mitogenesis to increased calcium and CD3 transduction of signal. Oettengen (4) speculated that one of the 3 chains of the CD3 complex was the actual calcium channel and that the antigen-bound TCR served to activate this calcium channel. He also showed that OKT3 induced an intracellular increase in calcium which was dependent upon extracellular calcium, was sensitive to membrane polarization, and was blocked by lanthanum, a calcium channel blocker.

Calcium Channel Blockers - Inhibition of T Cell Activation

Several studies have examined whether calcium channel blockers are effective in inhibiting T cell activation. Gearing (90),

using 3H thymidine uptake as measure of proliferation in rat blast cells and in a murine T cell clone, demonstrated that both verapamil and nifedipine at doses of 10 ug/ml inhibited IL-2 induced proliferation. Grier(91), using con A to stimulate bovine retropharyngeal lymphocytes, showed that verapamil inhibited 3H thymidine incorporation in a dose dependent manner (20% at 2 uM, 40% at 10 uM, and 60% at 20 uM). Another study, by McMillan(92), showed, that at doses more than 10 uM, verapamil inhibited murine T cell proliferation to alloantigen and con A.

Other studies, using peripheral blood mononuclear cells from healthy volunteers, have analyzed the effects of calcium channel blockers on human T cell activation. Larson (93) showed that verapamil at 10 ug/ml inhibited IL-2 induced 3H thymidine uptake by 20%, whereas the maximal inhibition was seen at 60 ug/ml. Walz (94), using phytohemagglutinin and phorbol myristate acetate as mitogens, demonstrated that verapamil required levels of at least 30 uM to have inhibiting effects on 3H thymidine uptake, calcium influx, and levels of IL-2 mRNA. Birx(95) determined the dose of three calcium channel blockers required to produce a 50% inhibition to con A (verapamil at 15 uM, nifedipine at 24uM, and diltiazem at 80 uM). Furthermore, Weir (96), varying doses of verapamil at 0.5 uM, 5 uM and 50 uM, demonstrated an inhibition of mRNA production at 3%, 24% and 84%, respectively, and an inhibition of new protein synthesis at 0%, 14% and 61%, respectively.

Therefore, it appears that calcium channel blockers inhibit T cell mitogenesis in vitro in a dose dependent manner. The

therapeutic plasma concentration for each of the three calcium channel blockers (verapamil, nifedipine, and diltiazem) is less than 200 ng/ml, or less than 0.5 uM (94,97,99-103). This level is less than the in vitro level shown in the previous studies to inhibit T cell mitogenesis. Weir (97) addressed the question of whether OKT3 stimulation of T cells was similarly affected. He incubated a culture of peripheral blood mononuclear cells from healthy adult donors with 3 ug/ml of OKT3 for 3 days. In a dose dependent fashion, verapamil inhibited OKT3 induced T cell proliferation by 22% at 0.5 uM, 30% at 5 uM, and 76% at 50 uM.

Madreoli (98) studied the immunological status of renal graft recipients who received calcium channel blockers for one month. The patients chosen for the study had shown no signs of rejection, nephrotoxicity nor viral infection and had been on an immunosuppressive therapy consisting of cyclosporine. He suggested that calcium channel blockers inhibited the mitogenic response to PHA and promoted an increase in CD8+ cells. More importantly, he used therapeutic doses of Nicardipine (60mg/day) and Diltiazem (90mg/day). Because the calcium channel blockers caused no change in the non calcium dependent poke weed mitogen, he concluded that calcium channel blockers work by blocking a calcium channel necessary for T cell activation. Madreoli implied that calcium channel blockers may inhibit T cell activation at normal therapeutic doses.

Purpose

To determine whether clinically used doses of calcium channel blockers are effective in ameliorating first dose reactions to OKT3.

Methods

A retrospective study was performed on all renal transplant patients who had received OKT3 between December 1983 (when OKT3 became available) and July 1990 (when this analysis began). Transplantation flow sheets (a daily record of a patient's hospital course) and discharge summaries were used to determine which patients had received OKT3 and the reason for its use. The patient population is described in Table 1. Pediatric cases were excluded from the study because this patient group may not reliably report symptoms.

The patients received OKT3 either to treat resistant rejection (19 in calcium channel blocker group vs 42 in control) or to avoid cyclosporine toxicity in the setting of delayed graft functioning (1 in calcium channel blocker group versus 7 in control). Administration of OKT3 (5mg/day intravenously) was preceded 30 minutes by acetaminophen (650 mg), diphenhydramine (25-50 mg), and corticosteroids. Some patients (N=31) received hydrocortisone 100 mg 30 minutes after OKT3. Because of the incidence and severity of first dose reactions, a physician administered the OKT3 while a nurse monitored for a reaction.

The hospital records were studied for demographic information, including age, sex, race, days of OKT3 therapy, and days post-op from transplant until OKT3 was given. Dialysis was defined by the patient undergoing dialysis (hemo or peritoneal) 1 day prior to OKT3 or up to 2 days after OKT3 was begun. Concomitant immunosuppressives, including azathioprine, cyclosporine, and

glucocorticoids, were recorded. Glucocorticoid doses were converted to methyl prednisolone equivalents using the following formula (4mg methyl prednisolone = 5 mg prednisone = 20 mg hydrocortisone).

The signs and symptoms of first dose reactions following the first 48 hours of OKT3 were recorded for each patient, and the definitions used are included in Table 2. The signs and symptoms analyzed in this study were chosen for their generally accepted occurrence after the first dose of OKT3 in studies that included more than 30 patient episodes (20,22,25,27,34,38,43,44).

Most of the information was obtained from the progress notes of the attending surgeons, the residents, and the nurses. Presence of fever, changes in blood pressure, tachycardia, emesis, and diarrhea were also checked on the nurses flow sheets. Trough blood levels of cyclosporine were obtained from a Chemistry Lab Data Sheet. The actual dosage and timing of all the medications were determined from the nursing record of drug administration.

The data were then analyzed to determine whether first dose reactions were less severe in those on calcium channel blockers (N=20) versus those off (N=49). To be included in the calcium channel blocker group, a patient had to be on calcium channel blockers for at least 3 days prior to the initiation of OKT3. Patients were receiving calcium channel blockers for the treatment of hypertension and angina. These patients did not receive calcium channel blockers for the treatment of rejection or for the prevention of reaction to OKT3. The number who received each type of calcium

channel blocker, the mean dose, and the range of doses are as follows : (Nifedipine : N=13, mean dose 66.2mg, range 30-120mg; Verapamil : N=5, mean 248mg, range 120-480mg; Diltiazem : N=2, mean 135mg, range 90-180mg). Patients receiving PRN nifedipine for hypertension (N=4) were not included in the calcium channel blocker group unless the total dose was greater than 30mg per day.

Statistics

All data were analyzed using chi-square analysis and Student T-tests with a SAS statistical program. The cyclosporine doses were compared also with Wilcoxon Rank-Sum test. These statistical tools were employed after consultation with bio-statisticians from Yale University School of Epidemiology and Public Health.

Results

At the time of OKT3 administration, 20 patients were on calcium channel blockers and 49 were not (Table 3). There were no significant differences between the two groups in age, sex, race, percent cadaveric transplants, and percent on dialysis. At the time of OKT3 administration, the dose and level of cyclosporine, the dose of azathioprine, and the dose of glucocorticoid were not different in the two groups.

Table 4 shows the percentages of the symptoms present in the two groups. Nearly 90% of each group developed fever. The peak temperature in the patients on calcium channel blockers was 102 $\pm$ 0.3°F (range 99.3-104.4) which was identical to the peak in those not on calcium channel blockers, 102 $\pm$ 0.2°F (range 98.8-105.0). Chills, gastrointestinal symptoms, respiratory complaints, and hemodynamic change were similar in both groups. Symptoms relating to lethargy and fatigue were the only ones that did differ between the two groups, occurring twice as frequently in the calcium channel blocker group.

Five patients received OKT3 on two different occasions, once on calcium channel blockers at the time OKT3 administration and once off. In general, each patient had similar reactions to the OKT3, regardless of presence of calcium channel blockers.

Discussion

A powerful immunosuppressant, OKT3 monoclonal antibody is used primarily to treat steroid resistant rejection or to protect the kidney from cyclosporine toxicity in the setting of post-transplant delayed graft functioning. The first and second doses of OKT3 are often accompanied by adverse reactions, ranging from a mild nuisance to life threatening sequelae. Various cytokines, namely TNF, IL-2, IFN, rise after OKT3 administration, in parallel with the appearance of the clinical syndrome. Because OKT3 has been shown to be a T cell mitogen, these cytokines are believed to be the product of T cell activation/proliferation, rather than T cell lysis. Calcium has been shown to be an important mediator of T cell activation after OKT3, and calcium channel blockers decrease T cell activation in vitro after stimulation with mitogens, including OKT3. This study attempted to determine whether clinically used doses of calcium channel blockers could effectively decrease the symptoms associated with the use of OKT3.

The frequencies of signs and symptoms of first dose reaction to OKT3 observed in this study are similar to the frequencies found by other investigators (20,22,25,27,34,38,43,44). Even though patients were not previously randomized, patient demographics, including immunosuppressive regimens at the time of OKT3 administration, were similar in the two groups. This study demonstrated that calcium channel blockers apparently offer no protection against the first dose reaction of OKT3. Analysis of peak temperature showed no difference between the two groups.

The reason why calcium channel blockers caused more fatigue is not clear. All calcium channel blockers can cause fatigue, which is usually tolerated with subsequent doses. There was no reason to suspect an inherent difference in the two groups regarding perception of or reporting of fatigue. Furthermore, because 16 different symptoms/signs were analyzed with significance looked for at P=0.05 level, there is a high likelihood that one of the factors would be significant purely based on chance.

What are the possible explanations for the lack of a protective effect of calcium channel blockers against first dose OKT3 reactions? Firstly, clinically used doses of calcium channel blockers may not achieve high enough blood levels to inhibit OKT3 induced T cell activation. The therapeutic plasma concentration for each of the three calcium channel blockers (verapamil, nifedipine, and diltiazem) is less than 200 ng/ml, or less than 0.5 uM (94,97,99-103). In all the in vitro studies (90-95) which used mitogens other than OKT3, the levels of calcium channel blockers required to inhibit T cell proliferation were substantially higher than the normal therapeutic level of 0.5uM by 1 to 2 orders of magnitude (5uM to 50uM). Several studies (94,95) demonstrated no inhibition at 0.5 uM while the others did not test at concentrations this low. The only in vitro study using OKT3 as the T cell mitogen (96) did show a 22% inhibition of T cell activation at 0.5 uM of verapamil. However, it is possible that such a small degree of inhibition, if it also occurs in vivo, is not sufficient to decrease the signs and symptoms related to lymphokine release. Because our

patients had received typical doses of calcium channel blockers for at least three days prior to receiving OKT3, blood levels, although not directly measured, were assumed to be in the therapeutic range. In vitro studies using OKT3 as the T cell mitogen should be repeated to determine if levels of calcium channel blockers achieved therapeutically (ie. less than 200 ng/ml) inhibit T cell proliferation. As always, however, the in vitro work may not be directly applicable to clinical settings.

Not all calcium channels blockers possess the same qualities. Carteza (104) studied the effects of calcium channel blocker on in vivo delayed type hypersensitivity in mice. Sensitizing mice with the antigen oxazolone, he found that nifedipine had significant suppressing effects, verapamil had significant enhancing effects, and diltiazem had no effect. Because the total number of patients on calcium channel blockers in this present study was small, the effect of the three individual calcium channel blockers could not be analyzed separately.

As the study was retrospective, the patients were not randomized prospectively to calcium channel blockers. The patients received calcium channel blockers for the treatment of hypertension and angina. The patients did not receive the blockers for the treatment of rejection or for the protection against the first dose reaction to OKT3. Some patients in the control group who had angina or hypertension were treated with different agents. The data were not examined to determine if the underlying indication for calcium

channel blockers could be responsible for the lack of any observable differences.

In this study, five patients received OKT3 twice, once on calcium channel blockers and once off them. Albeit a small number to analyze, there was no discernable difference in the side effects for each individual patient for the two different episodes. For each patient, the second episode of OKT3 caused a reaction that was similar to the first episode. The individual results in these five patients, with each one serving as his/her own control, strongly support our final conclusion about the absence of protection with calcium channel blockers.

The number of patients in this study was relatively small. Only 69 patient episodes of OKT3 could be found over a 6.5 year span, with an uneven distribution (20 on calcium channel blockers vs 49 controls) between the two groups. Perhaps with a larger, more evenly distributed sample, a different result would bear out. However, the data does not suggest that there was even a tendency toward a less severe reaction in our patients on calcium channel blockers.

Our study was a retrospective one. Because of the small number of patients receiving calcium channel blockers over the relatively long time frame, we had to chose this method. However, because the nurses and/or the intern closely monitors the hemodynamics and the temperature during the first few hours and because more objective symptoms such as vomiting/diarrhea are generally recorded by nurses on a daily flow sheet, these symptoms

were likely to be adequately recorded. In addition, there was no reason to suspect observer or recorder bias in either group. Although a prospective study would have allowed more detailed analysis of each sign and symptom, it is likely that the final result would be the same.

Koch-Weser (105) has pointed out that adverse drug reactions can be very ambiguous events, promoting widely divergent responses from clinical pharmacologists. One must first determine if an actual drug reaction is the proper etiology of the symptoms (as opposed to an illness) and then determine which drug caused it. In our patient population, not only are multiple disease states present (transplant, post-op, hypertension, rejection, etc) but so are multiple drug regimens. However, because reaction to OKT3 occurs so frequently and with a recognizable clinical syndrome, OKT3 is probably responsible for the clinical syndrome experienced by our patients. Because OKT3 induced side effects occur only with the first doses, reproduction of the syndrome with OKT3 rechallenge is difficult. However, as mentioned previously, the 5 patients who had received OKT3 (while on calcium channel blockers and while off them) had similar reactions both times.

In order to improve upon both reproducibility and validity, it is important to use explicit criteria for inclusion of a sign or symptom(106-108). As noted in the methods, this study did use explicit criteria. However, the adverse drug reactions were determined by one person (should be multiple) and at one time (if same person, should be repeated at later date). Because the

incidence of side effects in this study is similar to that reported in other studies, it is likely that retrospective studies are a valid means of testing for OKT3 associated side effects if explicit criteria are used (105-108).

In summary, in our study, calcium blockers did not protect against first dose reactions to OKT3. The best explanation to account for this result is that clinically used calcium channel blockers do not attain a high enough serum level to inhibit OKT3 induced T cell activation.

References

1. Terhorst,C.; "Structure and Function of the T-Cell Receptor/T3 Complex"; Transplantation Proceedings;V18;1986;931-936.

2. Weiss,A., J. Imboden, D. Shoback, and J. Stobo; "Role of T3 Surface Molecules in Human T-cell Activation : T3-Dependent Activation Results in an Increase in Cytoplasmic Free Calcium"; Proc. Natl. Acad. Sci.; V81; 1984; 4169-4173.

3. Samelson,L., J. Harford, R. Schwartz, and R. Klausner; "A 20kDa Protein Associated with Murine T Cell Antigen Receptor is Phosphorylated in Response to Activation by Antigen or Concanavalin A"; Proc. Natl. Acad. Sci.; V82; 1985; 1969-1973.

4. Oettgen,H., C. Terhorst, L. Cantley, and P. Rosoff; "Stimulation of the T3-T Cell Receptor Complex Induces a Membrane-Potential-Sensitive Calcium Influx"; Cell; V40; 1985, 583-590.

5. Suthanthiran,M., M. Fotino, R. Riggio etal; "OKT3-Associated Adverse Reactions : Mechanistic Basis and Therapeutic Options"; American Journal of Kidey Diseases; V14 suppl 2;1989;39-44.

6. Chatenoud,L., C. Ferran, C. Legendre etal; "In vivo Cell Activation Following OKT3 Administration : Systemic Cytokine Release and Modulation by Corticosteroids"; Transplantation; V49; 990; 697-702.

7. Chatenoud,L., C. Ferran, A. Reuter etal; "Systemic Reaction to the Anti-T-Cell Monoclonal Antibody OKT3 in Relation to Serum Levels of Tumor Necrosis Factor and Interferon-γ"; New England Journal of Medicine (Letter); V320;1989;1420-1421.

8. Abramowicz,D., L. Schandene, M. Goldman etal; "Release of Tumor Necrosis Factor, Interleukin-2, and Gamma-Interferon in Serum

after Injection of OKT3 Monoclonal Antibody in Kidney Transplant Recipients"; Transplantation; V47;1989;606-608.

9. Kung,P., G. Goldstein, E. Reinherz etal; "Monoclonal Antibodies Defining Distinct Human T Cell Surface Antigen"; Science (Letter); V206;1979;347.

10. Todd,P. and R. Brogden; "Muromonab CD3 : A Review of its Pharmacology and Therapeutic Potential"; Drugs;V37;1989;871-899.

11. Goldstein,G.; "Monoclonal Antibody Specificity : Orthoclone OKT3 T-Cell Blocker"; Nephron;V46 suppl 1;1987;5-11.

12. Chatenoud,L., C. Ferran, and J.-F. Bach; "In vivo Use of OKT3 : Main Issues for Monitoring of Treated Patients"; Transplantation Proceedings;V22;1990;2605-2608.

13. Suthanthiran,M., M. Wiebe, and K. Stenzel; "Effect of Immunosuppressants on OKT3 Associated T Cell Activation : Clinical Applications"; Kidney International; V32;1987;362-367.

14. Gebel,H., L. Lebeck, S. Jensik etal; "Discordant Expression of CD3 and T-Cell Receptor Antigens on Lymphocytes from Patients Treated with OKT3"; Trans. Proc.;V21;1989;1745-1746.

15. Zlabinger,G., D. Maurer, W. Ulrich etal; "Immunologic Monitoring in OKT3-Treated Kidney Graft Recipients"; Trans. Proc.; V22; 1990; 1777-1778.

16. Caillot-Zucman,S., C. Legendre, L.-H. Noel etal; "In Situ Antigenic Modulation of Human Graft-Infiltrating T Cells is Induced by OKT3 Treatment"; Trans. Proc.; V22; 1990; 1782.

17. Chauhan, B., T. Mohanakumar, and M. W. Flye; "Immunohistological Analysis of T Lymphocyte Subpopulations in Needle Core Biopsies

from OKT3-Treated Renal Allograft Recipients"; Transplantation; V50; 1990; 1058-1060.

18. Landegren,U., U. Ramstedt, I. Axberg etal; "Selective Inhibition of Human T Cell Cytotoxicity at Levels of Target Recognition or Initiation of Lysis by Monoclonal OKT3 and Leu-2a Antibodies"; J. Exp. Med.; V155, 1982; 1579-1584.

19. van Seventer,G., K. Kuijpers, R. van Lier etal; "Mechanism of Inhibition and Induction of Cytolytic Activity in Cytotoxic T Lymphocytes by CD3 Monoclonal Antibodies"; Journal of Immunolgy; V139; 1987; 2545-2550.

20. Cosimi,A. B., R. Burton, R. Colvin etal; "Treatment of Acute Renal Allograft Rejection with OKT3 Monoclonal Antibody"; Transplantation; V32 1981; 535-539.

21. Ortho Multicenter Transplant Study Group; "A Randomized Clinical Trial of OKT3 Monoclonal Antibody for Acute Rejection of Cadaveric Renal Transplants"; New England Journal of Medicine; V313; 1985; 337-342.

22. Norman,D., C. Shield, J. Barry etal; "Therapeutic Use of OKT3 Monoclonal Antibody for Acute Renal Allograft Rejection"; Nephron; V46 supp 1; 1987; 41-47.

23.Deierhoi,M., W. H. Barber, J. Curtis etal; "A Comparison of OKT3 Monoclonal Antibody and Corticosteroids in the Treatment of Acute Renal Allograft Rejection"; American Journal of Kidney Diseases; V11; 1988; 86-89.

24. Norman,D., J. Barry, W. Bennett etal; "The Use of OKT3 in Cadaveric Renal Transplantation for Rejection that is Unresponsive

to Conventional Anti-Rejection Therapy"; American Journal of Kidney Diseases; V11; 1988; 90-93.

25. Kahana,L., J. Ackermann, W. Lefor etal; "Uses of Orthoclone OKT3 for Prophylaxis of Rejection and Induction in Initial Nonfunction in Kidney Transplantation"; Trans. Proc.; V22; 1990; 1755-1758.

26. Kahana,L., and J. Baxter; "OKT3 Rescue in Refractory Renal Rejection"; Nephron; supp 1; 1987; 34-40.

27. Gordon, R., T. Starzl, J. Fung etal; "Monoclonal Antibody Therapy with Ciclosprin and Steroids in Nonmatched Cadaveric Renal Transplants"; Nephron; supp 1; 1987; 56-59.

28. Thistlewaite,J. R., J. Stuart, J. Mayes; "Use of a Brief Steroid Trial Before Initiating OKT3 Therapy for Renal Allograft Rejection"; American Journal of Kidney Diseases; V11; 1988; 94-98.

29. Fung,J., A. J. Demetris, K. Porter etal; Use of OKT3 with Ciclosporin and Steroids for Reversal of Acute Kidney and Liver Allograft Rejection"; Nephron; supp 1; 1987; 19-33.

30. Burdick,J., L. Pennington, W. Smith; "Reversal of Progressive Renal Allograft Dysfunction with OKT3"; Nephron; supp 1; 1987; 52-55.

31. Cohen,D., A. Benvenisty, J. Cianci, and M. Hardy; "OKT3 Prophylaxis in Cadaveric Kidney Transplant Recipients with Delayed Graft Functioning"; American Journal of Kidney Diseases; V14; 1989; 19-27.

32. Hricik,D., J. Zarconi, and J. Schulak; "Concomitant Low-Dose Cyclosporine and OKT3 Therapy for Renal Transplant Rejection"; Trans. Proc.; V21; 1989; 1755-57.

33. Hricik,D., J. Zarconi, and J. Schulak; "Influence of Low-Dose Cyclosporine on the Outcome of Treatment with OKT3 for Acute Renal Allograft Rejection"; Transplantation; V47; 1989; 272-77.

34. Kreis,H., N. Chkoff, L. Chatenoud etal; "A Randomized Trial Comparing the Efficacy of OKT3 Used to Prevent or to Treat Rejection"; Trans. Proc.; V21 Supp 2; 1989; 3-6.

35. Monaco, A.P.; "Renal Prophylaxis with Orthoclone OKT3 in the United States"; Trans. Proc.; V21 Supp 2; 1989; 7-13.

36. Shield, C.F.; "Use of OKT3 as Prophylaxis in Cadaveric Renal Transplantation"; Trans. Proc.; V21 Supp 2; 1989; 15-18.

37. Norman,D., C. Shield, J. Barry etal; "Early Use of OKT3 Monoclonal Antibody in Renal Transplantation to Prevent Rejection"; American Journal of Kidney Diseases; V11; 1988; 107-110.

38. Kahana, L., J. Narvarte, J. Ackermann etal; "OKT3 Prophylaxis versus Conventional Drug Therapy : Single-center Perspective, Part of a Multicenter Trial"; American Journal of Kidney Diseases; V14 Supp2; 1989; 5-9.

39. Schroeder,T., M. R. First, M. Mansour etal; Prophylactic Use of OKT3 in Immunologic High-risk Cadaver Renal Transplant Recipients"; American Journal of Kidney Diseases; V14 Supp 2; 1989; 14-18.

40. Light,J., N. Khawand, A. Aquino etal; "Quadruple Immunosuppression : Comparison of OKT3 and Minnesota Antilymphocyte Globulin"; American Journal of Kidney Diseases; V14 Supp 2; 1989; 10-13.

41. First, M. R., T. Schroeder, P. Hurtubise etal; "Successful Retreatment of Allograft Rejection with OKT3"; Transplantation; V47; 1989; 88-91.

42. Mayes,J., J. R. Thistlewaite, J. Stuart etal; "Reexposure to OKT3 in Renal Allograft Recipients"; Transplantation; V45; 1988; 349-353.

43. Thistlewaite,J. R., J. Stuart, J. Mayes; "Complications and Monitoring of OKT3 Therapy"; American Journal of Kidney Diseases; V11; 1988; 112-119.

44. Sumrani,N., V. Delaney, D. Rajpoot etal; "OKT3 in Severe Early Rejection : Predictors for Reversal in Renal Transplant Recipients"; Trans. Proc.; V22; 1990; 1750-1752.

45. Martin,M., R. M. Massanari, D. Nghiem etal; "Nosocomial Asceptic Meningitis Associated with Administration of OKT3"; JAMA; V259; 1988; 2002-2005.

46. Rosenberg,S., M. Lotze, L. Muul etal; "A Progress Report on the Treatment of 157 Patients with Advanced Cancer Using Lymphokine-Activated Killer Cells and Interleukin-2 or High-Dose Interleukin-2 Alone"; New England Journal of Medicine; V316; 1987; 889-897.

47. Remick,D., R. Kunkel, J. Larrick, and S. Kunkel; "Acute in Vivo Effects of Human Recombinant Tumor Necrosis Factor"; Laboratory Investigation; V56; 1987; 583-590.

48. Beutler,B. and A. Cerami; "Cachectin (Tumor Necrosis Factor) : A Macrophage Hormone Governing Cellular Metabolism and Inflammatory Response"; Endocrine Reviews; V9; 1988; 57-66.

49. Kinkhabwala,M., P. Sehajpal, E. Skolnik etal; " A Novel Addition to the T Cell Repertory : Cell Surface Expression of Tumor Necrosis Factor/Cachectin by Activated Normal Human T Cells"; J. Exp. Med.; V171; 1990; 941-946.

50. Bloemena,E., I. Ten Berge, J. Surachno, and J. Wilmink; "Kinetics of Interleukin 6 During OKT3 Treatment in Renal Allograft Recipients"; Transplantation; V50, 1990; 330-331.

51. Rao,K.V.; "Mechanism, Pathophysiology, Diagnosis, and Management of Renal Transplant Rejection"; Medical Clinics of North America; V74; 1990; 1039-57.

52. Suthanthiran,M., W. Kaye, A. Rubin, and K. Stenzel; "Interleukin-2 Profiles in Renal Graft Recipients"; Trans. Proc.; V16; 1984; 1473-74.

53. Maury,C.; "Monitoring the Acute Phase Response"; Journal of Clinical Pathology; V43; 1989; 1078-82.

54. Maury,C., and A.-M. Teppo; "Raised Serum Levels of Cachectin/Tumor Necrosis Factor α in Renal Allograft Rejection"; J. Exp. Med.; V166; 1987; 1132-37.

55. Patel,S., A. Duby, D. Thiele, and P. Lipsky; "Phenotypic and Functional Characterization of Human T Cell Clones"; Journal of Immunology; V141; 1988; 3726-36.

56. Grabstein,K., S. Dower, S. Gillis etal; "Expression of Interleukin 2, Interferon-γ, and the IL 2 Receptor by Human Peripheral Blood Lymphocytes"; Journal of Immunology; V136; 1986; 4503-8.

57. Stohl,W., Z. Tovar, and N. Talal; "Generation of Cytolytic Activity with Anti-CD3 Monoclonal Antibodies Involves both IL-2-

Independent and -Dependent Components"; Journal of Immunology; V144; 1990; 3718-25.

58. Sherris,D., W. Stohl, and L. Mayer; "Characterization of Lymphokines Mediating B Cell Growth and Differentiation from Monoclonal Anti-CD3 Antibody-Stimulated T Cells"; Journal of Immunology; V142; 1989; 2343-51.

59. Norman,D.; "An Overview of the Use of the Monoclonal Antibody OKT3 in Renal Transplantation"; Trans. Proc.; V20; 1988; 1248-1252.

60. Suranyi,M., and B. Hall; "Renal Transplantation"; The Western Journal of Medicine; V152; 1990; 689-690.

61. Cosimi,A. B.; "OKT3 : First-Dose Safety and Success"; Nephron; V46 Supp 1; 1987; 12-18.

62. Delmonico,F., and A. B. Cosimi; "Monoclonal Antibody Treatment of Human Allograft Recipients"; Surgery, Gynecology, and Obstetrics; V166; 1988; 89-98.

63. van Wauwe,J., J. de Mey, and J. Goosens; "OKT3 : A Monoclonal Anti-Human T Lymphocyte Antibody with Potent Mitogenic Properties"; Journal of Immunology; V124; 1980; 2708-13.

64. von Wussow,P., C. Platsoucas, M. Wiranowska-Stewart, and W. Stewart; "Human γ Interferon Production by Leukocytes Induced with Monoclonal Antibodies Recognizing T Cells"; Journal of Immunology; V127; 1981; 1197-1200.

65. van Wauwe,J., J. Goosens and P. Beverly; "Human T Lymphocyte Activation by Monoclonal Antibodies : OKT3, but not UCHT1, Triggers Mitogenesis via an Interleukin 2-Dependent Mechanism"; Journal of Immunology; V133; 1984; 129-132.

66. Turner,M., M. Londei and M. Feldman; "Human T Cells from Autoimmune and Normal Individuals can Produce Tumor Necrosis Factor"; Eur. J. Immunology; V17; 1987; 1807-1814.

67. Ellenhorn,J., E. S. Woodle, I. Ghobreal etal; "Activation of Human T Cells in Vivo Following Treatment of Transplant Recipients with OKT3"; Transplantation; V50; 1990; 608-612.

68. Smith,K., J. Austyn, G. Hariri etal; "T Cell Activation by anti-T3 Antibodies"; Eur. J. Immunology; V16; 1986; 478-486.

69. Chang,T.-W., D. Testa, P. Kung etal; "Cellular Origin and Interactions Involved in γ-Interferon Production Induced by OKT3 Monoclonal Antibody"; Journal of Immunology; V128; 1982; 585-89.

70. Ceuppens,J., L. Meurs and J. van Wauwe; "Failure of OKT3 Monoclonal Antibody to Induce Lymphocyte Mitogenesis : A Familial Defect in Monocyte Helper Function"; Journal of Immunology; V134; 1985; 1498-1502.

71. Thistlewaite,J.R., T. Heffron, J. Stuart etal; "Selective OKT3 Induction Therapy in Adult Cadaveric-Donor Renal Transplant Recipients"; American Journal of Kidney Disease; V14 Supp 2; 1989; 28-34.

72. Kirkpatrick,C.; "Transplantation Immunology"; JAMA; V258; 1987; 2993-3000.

73. Monaco,A.; "Immunosuppression and Renal Transplantation"; Nephron; V46 Supp 1; 1987; 1-4.

74. Granelli-Piperno,A., L. Andrus, and R. Steinman; "Lymphokine and Nonlymphokine mRNA Levels in Stimulated Human T Cells"; J. Exp. Med.; V163; 1986; 922-37.

75. Tilney,N.; "Renal Transplantation"; Current Problems in Surgery; V26; 1989; 606-669.

76. Arya,S., F. Wong-Staal, and R. Gallo; "Dexamethasone-Mediated Inhibition of Human T Cell Growth Factor and γ-Interferon Messenger RNA"; Journal of Immunology; V133; 1984; 273-76.

77. Kelso,A., and A. Munck; "Glucocorticoid Inhibition of Lymphokine Secretion by Alloreactive T Lymphocyte Clones"; Journal of Immunology; V133; 1984; 784-791.

78. Gaston,R., M. Deierhoi, T. Patterson etal; "OKT3 First-Dose Reaction : Association with T Cell Subsets and Cytokine Release"; Kidney International; V39; 1991; 141-48.

79. Leo,O., M. Foo, D. Sachs etal; "Identification of a Monoclonal Antibody Specific for a Murine T3 Polypeptide"; Proc. Natl. Acad. Sci.; V84; 1987; 1374-78.

80. Hirsch,R., M. Eckhaus, H. Auchincloss etal; "Effects of in vivo Administration of Anti-T3 Monoclonal Antibody on T cell Function in Mice : Immunosuppression of Transplantation Responses"; Journal of Immunology; V140; 1988; 3766-72.

81. Hirsch,R., R. Gress, D. Pluznik etal; "Effects of in vivo Administration of Anti-T3 Monoclonal Antibody on T cell Function in Mice : In Vivo Activation of T Cells"; Journal of Immunology; V142; 1989; 737-43.

82. Ferran,C., K. Sheenan, M. Dy etal; "Cytokine-related Syndrome Following Injection of Anti-CD3 Monoclonal Antibody : Further Evidence for Transient in vivo T Cell Activation"; Eur. J. Immunolgy; V20; 1990; 509-15.

83. Alegre,M., M. Depierreux, S. Florquin etal; "Acute Toxicity of Anti-CD3 Monoclonal Antibody in Mice : A Model for OKT3 First Dose Reactions"; Trans. Proc.; V22; 1990; 1920-21.

84. Ferran,C., J. Bluestone, J.-F. Bach, and L. Chatenoud; "In vivo T Lymphocyte Activation Induced in Mice Following the Injection of Anti-CD3 Monoclonal Antibody"; Trans. Proc.; V22; 1990; 1922-23.

85. Ferran,C., M. Dy, S. Merite etal; "Reduction of Morbidity and Cytokine Release in Anti-CD3 MoAb-Treated Mice by Corticosteroids"; Transplantation; V50; 1990; 642-8.

86. Tsien,R., T. Pozzan, and T. Rink; "T Cell Mitogens Cause Early Changes in Cytoplasmic Free Calcium and Membrane Potential in Lymphocytes"; Nature; V295; 1982; 68-70.

87. Lichtman,A., G. Segal, and M. Lichtman; "The Role of Calcium in Lymphocyte Proliferation (An Interpretive Review)"; Blood; V61; 1983; 413-422.

88. Mills,G., R. Cheung, S. Grinstein, and E. Gelfand; "Increase in Cytosolic Free Calcium Concentration is an Intracellular Messenger for the Production of Interleukin 2 but not for Expression of the Interleukin 2 Receptor"; Journal of Immunology; V134, 1985; 1640-43.

89. Mills,G., R. Cheung, S. Grinstein, and E. Gelfand; "Interleukin 2-Induced Lymphocyte Proliferation is Independent of Increases in Cytosolic-Free Caclium Concentrations"; Journal of Immunology; V134, 1985; 2431-35.

90. Gearing,A., M. Wadhwa, and A. Perris; "Interleukin 2 Stimulates T Cell Proliferation Using a Calcium Flux"; Immunology Letters; V10; 1985; 297-302.

91. Grier,C., and A. Mastro; "Mitogen and Co-Mitogen Stimulation of Lymphocytes Inhibited by Three Calcium Antagonists"; Journal of Cellular Physiology; V124; 1985; 131-136.

92. McMillen,M., T. Lewis, B. Jaffe, and R. Wait; "Verapamil Inhibition of Lymphocyte Proliferation and Function in Vitro"; Journal of Surgical Research; V39; 1985; 76-80.

93. Larsen,C., T. Knudsen, and H. Johnson; "The Role of Calcium in Stimulation of Activated T Lymphocytes with Interleukin 2"; Scand. J. Immunol.; V24; 1986; 689-697.

94. Walz,G., B. Zanker, K. Weider etal; "Similar Effects of Cyclosporine and Verapamil on Lymphokine, Interleukin 2 Receptor, and Proto-oncogene Expression"; Transplantation; V47, 1989; 331-34.

95. Birx,D., M. Berger, and T. Fleisher; "The Interference of T Cell Activation By Calcium Channel Blocking Agents"; Journal of Immunology; V133; 1984; 2904-09.

96. Weir,M., R. Peppler, D. Gomolka, and B. Handwerger; "Additive Inhibitory Effect of Cyclosporine and Verapamil May Occur Through Different Mechanisms that May Be Dependent or Independent of the Slow Calcium Channel"; Trans. Proc.; V21; 1989; 866-870.

97. Weir,M., R. Peppler, D. Gomolka, and B. Handwerger; "Additive Effect of Cyclosporine and Verapamil on the Inhibition of Activation

and Function of Human Peripheral Blood Mononuclear Cells"; Trans. Proc.; V20 Supp 2; 1988; 240-244.

98. Madreoli,M., E. D. Esposti, R. Cocchi etal; "Do Calcium Channel Blockers have any Influence on the Immunological Status of Renal Graft Recipients on Ciclosporin Therapy"; American Journal of Nephrology; V10; 1990; 58-60.

99. Frishman,W., J. Stroh, S. Greenberg etal; "Calcium Channel Blockers in Systemic Hypertension"; Medical Clinics of North America; V72; 1988; 449-96.

100. Frohlich,E.; "Clinical Pharmacology of Calcium Antagonists : Satellite Symposium on Calcium Antagonists"; Hypertension; V11 Supp. I; 1988; 222-24.

101. McAllister,R., S. Hamann, and R. Blouin; "Pharmacokinetics of Calcium Entry Blockers"; American Journal of Cardiology; V55; 1985; 30B-40B.

102. Freedman,S.B., D. Richmond, J. Ashley, and D. Kelley; "Verapamil Kinetics in Normal Subjects and Patients with Coronary Artery Spasm"; Clin Pharm Therapeutics; V30; 1981; 644-52.

103. Kleinbloesem,C., P. van Brummelen, H. Faber, and D. Breimer; "Pharmacokinetics and Hemodynamic Effects of Long-Term Nifedipine Treatment in Hypertensive Patients"; Journal of Cardiovascular Pharmacology; V9; 1987; 202-208.

104. Corteza,Q., S. Shen, D. Revie, and P. Chretien; "Effects of Calcium Channel Blockers on in Vivo Cellular Immunity in Mice"; Transplantation; V47; 1989; 339-342.

105. Koch-Weser,J., E. Sellers, and R. Zacest; "The Ambiguity of Adverse Drug Reactions"; European Journal of Clinical Pharmacology; V11; 1977; 75-78.

106. Boyd,N., J. Pater, A. Ginsburg, and R. Myers; "Observer Variation in the Classification of Information from Medical Records"; Journal of Chronic Diseases; V32; 1979; 327-332.

107. Hutchinson,T., J. Leventhal, M. Kramer etal; "An Algorithm for the Operational Assessment of Adverse Drug Reactions : Demonstration of Reproducibility and Validity"; JAMA; V242; 1979; 633-638.

108. Leventhal,J., T.Hutchinson, M. Kramer, and A. Feinstein; "An Algorithm for the Operational Assessment of Adverse Drug Reactions : Results of Tests Among Clinicians"; JAMA; V242; 1979; 1991-94.

Table 1 Patient Population

Category	Number of Episodes
Transplant recipients (1983-1990)	224
OKT3 episodes(a)	80
Files not used(b)	-11
OKT3 episodes in study	69
Episodes on calcium channel blockers	20
Episodes not on calcium channel blockers	49

(a) An episode of OKT3 use (to treat rejection or used prophylactically to prevent cyclosporine toxicity in the early post transplant period)

(b) 2 files not found, 1 confidential file, 5 pediatric cases, 3 pancreas/kidney recipients

Table 2 Definitions of Signs and Symptoms

Fever - Temperature > 100 F in first 48 hours with negative cultures
Chills - Complaint of chills, rigors
Dyspnea - Complaint of shortness of breath, trouble breathing
Wheeze - Complaint of wheeze, or noticed on physical exam
Chest pain - Complaint of chest pain or chest tightness
Emesis - Presence of vomiting
Diarrhea - Presence of diarrhea
Headache - Presence of headache
Seizure - New onset of seizure and witnessed by med staff
Malaise - Complaint of lethargy, extreme fatigue
Aseptic meningitis - Lumbar puncture showing white blood cells in cerebral spinal fluid with negative cultures
Tachycardia - Heart rate > 100
Hypotension - Systolic < 90 or decrease in systolic > 20 mm Hg
Hypertension - Systolic > 160 or increase in systolic > 20 mm Hg
Arthralgia - Complaint of joint aches/pains
Myalgia - Complaint of muscle aches/pains

Table 3 Comparison of Demographic Data

Variable	Calcium Channel Blocker (N=20)	Control (N=49)
Sex (% Male)	51	70
Age (years) (a)	37.3 ± 2.9	36.8 ± 1.8
Race (% Black)	15	18
Transplant Type (% cadaver)	90	78
Dialysis (b)	45	49
Days post transplant (c,d)	27 (0-1874)	21 (0-995)
CSA Dose (mg/day)- Day Before (a)	485.5 ± 87	342.2 ± 61
CSA Level (ng/ml)- Day Before (a)	118.3 ± 33	81.8 ± 15
CSA Dose (mg/day)- Day of OKT3 (a)	390.0 ± 81	239.0 ± 49
CSA Level (ng/ml)- Day of OKT3 (a)	124.7 ± 31	114.8 ± 21
Imuran dose (mg) (a)	11.8 ± 6.8	17.3 ± 6.0
Solumedrol dose (mg) (24 hour before OKT3) (a)	301.9 ± 90	271.1 ± 34
Solumedrol dose (mg) (1 hour before OKT3) (a)	104.4 ± 31	134.3 ± 25

a Mean ± standard error of mean
b Percent of patients on dialysis
c Days after transplant when OKT3 was started
d Median (Range)

Table 4 Signs and Symptoms of First Dose Reaction to OKT3(a)

	Calcium Channel Blocker (N=20)	Control (N=49)
Sign/Symptom(b)	Percent(c)	Percent(c)
Fever	90	88
Malaise	70 *	33
Chills	60	41
Tachycardia	50	51
Emesis	50	35
Hypertension	45	45
Diarrhea	40	45
Myalgia	35	16
Hypotension	30	23
Arthralgia	30	16
Headache	25	43
Dyspnea	25	23
Wheeze	20	8
Chest Pain/Tightness	15	14
Aseptic Meningitis	10	4
Seizure	0	0

* $P < 0.005$ by chi-square analysis
a Within first 48 hours
b Criteria for each sign/symptom is listed in Table 2
c Percent in each group with a given sign or symptom

HARVEY CUSHING / JOHN HAY WHITNEY
MEDICAL LIBRARY

MANUSCRIPT THESES

Unpublished theses submitted for the Master's and Doctor's degrees and deposited in the Medical Library are to be used only with due regard to the rights of the authors. Bibliographical references may be noted, but passages must not be copied without permission of the authors, and without proper credit being given in subsequent written or published work.

This thesis by has been used by the following persons, whose signatures attest their acceptance of the above restrictions.

NAME AND ADDRESS DATE