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Functions and Interrelationships of Leukocytes in Inflammation as Elucidated by the Rebuck Skin Window Technique

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This article provides an overview of the functions of human leukocytes in inflammation as elucidated by the Rebuck skin window technique. The migration sequence of various leukocytes into the field of inflammation is described, as well as cytologic, cytochemical, and transformational changes, and the interrelationships of responding leukocytes. Since it was introduced in 1955, the Rebuck skin window technique has provided an excellent means of studying in vivo inflammatory response to different phlogistic agents in normal individuals and in various disease states. This simple technique continues to prove fruitful to further study and to monitoring of many disease states.

Since detailed descriptions of the stages of inflammation can be found in numerous texts (1-6), this article will present an overview of leukocytic migration, morphologic changes, and functions as elucidated by the Rebuck skin window technique (7-9) in normal subjects as well as in various disease states.

The classic article by Rebuck and Crowley (7) describing their simple yet definitive method of serially studying the exudative response to phlogistic agents is reproduced in this section (pp. 184-209). Since its publication, this reproducible and inexpensive method has been extensively used (9). It has allowed monitoring of leukocytic reactions and functions in response to many different inflammatory excitants in the normal individual as well as in several disease states. Over the years, it has undergone few alterations and, if anything, has become simpler to use because of the availability of chemically and antigenically inert sterile plastic cover slips, hypoallergenic adhesive tape, etc. Also, modifications of the scarification technique and semiautomated mechanical means of scarification have been introduced (10-16) that may provide standardization in the hands of the inexperienced. However, the original scarification procedure is quite easy to learn and reproduce with minimal experience.

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Reaction to Trauma of the Technique

Mechanical trauma produced by scarification alone could be expected to produce hyperemia and prestasis as factors for increasing vascular permeability (7,18). In most types of injury, biphasic permeability has been demonstrated (19-21); an early but transient phase is followed by a variable latent period and a late prolonged phase of increased permeability. The increase in permeability is due mainly to histamine release (22) and to the action of other vasoactive peptides and kinins (23-38). Depending upon the type, extent, and severity of the injury, the amount of plasma and leukocyte migration through the gaps between the endothelial cells (39,40) varies considerably. However, the earliest responding cells are almost always polymorphonuclear neutrophilic granulocytes (PMNs) (21,-30,31). Their emigration by active ameboid movement from the intravascular areas to the site of inflammation has been fairly well established (1,40-51). The skin window technique has repeatedly substantiated PMN migration as an early event (7,8,52). At the same time it is important to remember that the trauma of the technique alone produces only sparse leukocytic response consisting of a few neutrophils, a rare lymphocyte, a few local tissue macrophages, and a few hematogenous monocytes.

Leukocytic Response to Antigenic Exposure

The inflammatory response to an antigen in an individual without prior exposure should be related to its local phlogistic characteristics without the mediation of antigen-antibody reactions, complement activation, etc. Hurley

(40) has shown that injecting inert substances produces no increase in vascular permeability but causes delayed leukocytic migration. However, injecting histamine or serum causes both increased vascular permeability and delayed cell migration. The skin window technique demonstrates that applying diptheria-tetanus toxoid (DT 0.5m, Lilley) to the scarified skin of nonimmunized individuals induces cell migration and changes comparable to what could be expected by the mechanical trauma of the technique alone (9). However, since most individuals in the U.S. are immunized to DT, dynamic leukocytic response is elicited when DT is applied to the scarified area of most individuals.

Many articles by Rebuck and others (7,8,52) have provided descriptions of the leukocyte types, numbers, morphologic changes, etc. Recently, Sokol, Durrant, and Hudson (53) have presented information gained by scanning electron microscopy of skin window cells from normal subjects. In brief, the first wave of emigrating cells are PMNs, blood monocytes, and occasional lymphocytes. The number of emigrating monocytes is generally comparable to the numbers encountered in the blood stream. Although the number of emigrating PMNs decreases with the timing of the sample in 6-24 hour cover slip preparations, they show dynamic cytoplasmic and nuclear changes. PMN nuclei become progressively hypersegmented with 1.3% of the cells having more than five lobes or up to ten at 10-12 hours of response. In the cytoplasm, glycogen synthesis increases and is later transferred to mononuclears for energization. Lipid content of the migrated PMNs is high, and most enzymatic reactions of PMNs continue to be enhanced until they lose their viability (7-9). The only known enzyme to diminish quickly in the PMNs is leukocyte alkaline phosphatase.

As mentioned, monocytes responding to inflammation are numerically equivalent to the numbers (percentages) in the blood (9). All authorities agree that blood monocytes are capable of transforming into macrophages at the inflammatory site (9,52,55). The 3-12 hour cover slip preparations have shown that the monocytes are metallophilic, concentrate dyes, and progressively demonstrate the cytochemical features of macrophages (8). Recent experiments indicate that mononuclear cells leave the vessels more or less simultaneously with PMN leukocytes but persist longer in the exudate because of their longer life span (56-58). Furthermore, macrophages originate predominantly from blood monocytes and not from tissue histiocytes (57,59).

The role of lymphocytes in acute inflammatory response and their capability to transform into macrophages is still fervently debated (7,8,52,56,57,60). Originally, Metchnikoff observed (61) that most macrophages are transformed lymphocytes. Experiments by Kolouch (62), Townsend and Campbell (63), Good (64), Berman (65), and many others (79,135) have provided conclusive support for lymphocyte to macrophage transformation as reported by Rebuck and Crowley (7). Subsequent application of the skin window technique in experimental inflammatory lesions in man has yielded confirmatory evidence that human lymphocytes are indeed capable of transforming into macrophages (55, 67-74). At three hours the emigrated lymphocytes in the skin windows are scanty. However, 9-14 hour preparations reveal that most of the responding mononuclear cells are small and medium-sized lymphocytes that slowly hypertrophy by increase in colorless cytoplasm, as well as by basophilic material in the enlarging cell body. An increase in the irregularity of the nuclear membrane and loosening of dense chromatin accompany hypertrophy. At 14-18 hours, further lymphocyte hypertrophy and other transformational changes lead to the formation of lymphocytogenous macrophages. Cytochemical analysis of the transformational process has shown that in addition to the presence of acid phosphatase and glycogen, which have been noted in the circulating lymphocyte, the transforming lymphocytes acquire such enzymes as oxidase, peroxidase, alkaline phosphatase, as well as sudanaphilic cytoplasmic constituents (9). These lymphocytes also become phagocytic for vital dyes, pyrrol blue, lithium-carmine, trypan blue and trypan red (8).

Improved knowledge of the lymphocyte physiology and functions allows us to recognize lymphocytes as B, T, null, and/or one of the subpopulations. It would appear natural that the T lymphocytes involved in cell-mediated immunity are the ones which would predominantly emigrate to inflammatory sites and then transform to macrophages. It has been shown that applying antithymocyte globulin (ALG) at the 10th hour of an antigenically stimulated skin window site completely eliminates the main mass of small round mononuclears by the succeeding 12th hour of inflammation (9). This not only confirms that most of the responding cells are T lymphocytes but also helps to differentiate further the lymphocytic from the monocytic source of macrophages.

Leukocytic Response in Disease States

Because it allows one to serially sample the exudative response to inflammatory stimuli, the skin window technique has provided an excellent means of studying leukocyte response in many disease states.

Decreased granulocyte emigration and/or function

Decreased emigration of PMNs in skin windows of patients with neutropenia due to varying causes was first reported by Riis (67). The close correlation he found between leukocytic response in skin windows and the inflammatory lesions in the organs of some autopsy patients has been

confirmed by Rebuck, et al (54). It is obvious that delayed or absent PMN response in windows has considerable significance when the capabilities of cellular defenses in disease states are to be correlated. Decreased granulocyte migration is also critical, since initial neutrophilic migration is an essential step leading to the migration of lymphocytes and their successful transformation to macrophages. Impaired neutrophilic response in skin windows has been reported in congenital neutropenia (75), neutrophil actin dysfunction (76), diabetes mellitus (77-79), paroxysmal nocturnal hemoglobinuria (54,80), Chediak-Higashi anomaly (81,82), complement component deficiences (83-85), leukemias and myeloproliferative disorders (9), established malignant states (9), alcoholism (86), burns (87,88), kala azar and Schistosomiasis mansonii (89), and conditions causing elevated serum levels of chemotactic factor inactivator (90). Decreased neutrophilic migration has also been observed in patients who are being treated with ACTH, hydrocortisone, progestron, prednisone, gold salts, chloropheniramine maleate, and imuran (9). Thus, neutropenic individuals are more susceptible to infections and show poor inflammatory response, but other conditions which impair PMN migration, release of hydrolytic enzymes (Metchnikoff's cytases) (61,91,92), and impairment of phagocytosis can also lead to reduced chemotaxis or transformation of mononuclears (48).

Skin window studies have revealed diminished phagocytic capabilities of neutrophils in Hegglins and Pelger Huet anomalies (93), paroxysmal nocturnal hemoglobinuria (54,80,93), chronic granulocytic leukemia (94), uveitis (95), during hydrocortisone or ACTH therapy (96), and when urea, urine (97-99), or crystals of gouty tophi (100) are used in skin windows.

Diseases associated with exuberant granulocyte emigration

An orderly, controlled response by blood and tissue elements is obviously necessary for proper healing of all injuries. Excessive cellular outpouring into the field of inflammation due to a chemotactic or phlogistic agent can be undesirable, as observed in the Schwartzman and the Arthus phenomena (101-103). Interaction of immune aggregates and the complement system releases substances that are strongly chemotactic for PMNs (104-106). Phagocytosis of immune aggregates leads to explosive release of lysosomal material with attendant hydrolysis and other changes (107-112). In allergic individuals, the antigen-antibody complexes are not only chemotactic for PMNs, but are also specifically chemotactic for eosinophils (113-116). It is hypothesized that eosinophils are attracted by allergic responses that promote fibrin formation since the specific eosinophil granules have been shown to contain profibrinolysin.

In the skin windows, excessive or persistent PMN migration has been noted in a few disease states. In patients with nonspecific uveitis, Hessburg and Rebuck (95) found that excessive persistent neutrophilic migration was not followed by normal mononuclear transformations. In these patients, uveal pigment placed at the test sites also resulted in excessive outpouring of eosinophils. In patients with untreated polycythemia vera, increased PMN emigration but decreased mononuclear emigration has also been observed (117).

When Waldmann, et al (118-119) discovered the Fitzgerald factor, they applied the skin window lesion to Mr. Fitzgerald, who lacked this coagulation factor (and after whom it was named). They observed a massive outpouring of PMNs which persisted through the 49th hour of study. Increased numbers of eosinophilic and basophilic granulocytes were also observed at various test intervals. They speculated that the exudative changes in this otherwise healthy individual compensated for the failure of surface-mediated generation of fibrinolytic activity.

While eosinophilic granulocytes normally comprise less than 0.1% of responding cells in the skin windows, markedly increased response in hypersensitive individuals has been reported using many different experiments (9). It has been described in antigen hypersensitivity of immediate type (120) but not in patients with delayed onset of food hypersensitivity. Excessive eosinophil migrations have been induced in windows of challenged, passively sensitized individuals, and Fowler and Lowell (113) have been able to correlate the eosinophilic response to clinical severity. The application of topical steroids fails to abolish eosinophilic response but oral steroid therapy, especially if administered daily, prevents it.

There are not many recognized diseases that affect basophil emigration or their function in inflammation. In a normal control window site the number of emigrating basophils is usually negligible, although the technique has focused specific attention on some disorders of basophilic granulocyte emigration and function. Basophilic granulocytes are the blood-borne counterpart of tissue mast cells and, like them, are a rich source of histamine, heparin, and chymase. Using the skin window technique, Priest and his associates (54,55,121-24) reported basophilic granulocyte outpouring in ulcerative colitis and postulated that the colonic and other lesions were due to abnormal basophil activity and release of excess histamine, heparin, and chymase. This concept is well supported by the fact that there is a significant increase of metachromatically granulated cells in the colonic lesions of ulcerative colitis. Similar abnormal basophilic migration has been seen in the windows of patients with interstitial cystitis (9,123), another disease with a high number of metachromatically-granul-

ated cells in the bladder wall. Abnormal basophil emigration has also been described in patients with furunculosis (125) and in histoincompatibility (126-28).

Diseases affecting monocyte response

Not too many conditions are known to cause lack of hematogenous monocyte response in skin windows. Even in immunosuppressed individuals whose granulocytic and lymphocytic response is below normal, the monocyte response remains reasonably constant unless oversuppression occurs. In chronic immunosuppression, whether induced, acquired or congenital, the monocyte response is increased in proportion to their increase in blood (9).

A very interesting and significant finding in the skin windows of patients with genetic mucopolysaccharidosis is the appearance of increased basophils and quasi-mast cells (123,129-31) due to the ingestion of metachromatic acid mucopolysaccharides by the macrophages. Since metachromatic quasi-mast cell macrophages regularly appear in the skin window, even in forme fruste cases (129), this technique could be helpful in evaluating suspected cases of genetic mucopolysaccharidosis.

Diseases affecting lymphocyte response

Since it is well established that neutrophilic appearance in normal numbers is essential for the emigration, energization, and transformation of lymphocytes to macrophages (9), any disorders which impair neutrophil emigration and release of lymphochemotactic substances will lead to decreased lymphocyte participation and function. For example, in the skin windows of patients with far advanced carcinomas, sarcomas, and Hodgkin's disease decreased lymphocyte response has been described (9).

Perhaps the lymphocytolytic and immunosuppressive agents, such as ACTH, cortisone, imuran, and other antimetabolites not only decrease lymphocyte participation by systemic action but also, as detailed above, by affecting the neutrophil emigration. On the other hand, rapid transformation of lymphocytes to macrophages in Boeck's sarcoid has been reported (9,132), although the exact cause of this rapid transformation is not apparent. It would appear that the disorders specifically involving T lymphocytes should affect the response of lymphocytes and their transformation in skin windows. Depressed cell-mediated immunity and decreased mononuclear response in skin windows have been observed in Darier's disease (133). However, many controlled studies for T cell function, migration, and transformation need to be performed and may provide a fruitful avenue of further research.

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