



The "Three R's" of Delayed Hypersensitivity*

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Delayed hypersensitivity is one of several immune responses initiated by thymus-derived (T) lymphocytes. Other functions of T cells are listed in Table 1 and summarized in reference 1. The mononuclear infiltrate of delayed hypersensitivity is a collaborative phenomenon between T lymphocytes and monocytes. This collaboration can be separated into components which I have chosen to call the "three R's" of delayed hypersensitivity. In this discussion I will define these "three R's" and examine the usefulness of this concept in clinical medicine.

Hematoxylin and eosin sections of skin biopsied 48 hours after intradermal injections of antigen characteristically show mononuclear cells around blood vessels and in connective tissue. As early as 1951, however, Gell and Hinde (2) reported that the monocyte was the principal participant in the skin site. In 1963, McCluskey (3), using radioisotopic techniques, found that 90% of the mononuclear cells in the reaction site were blood-borne and originated from cells that had divided 48 hours previously. Subsequently, cell transfer studies have provided conclusive evidence that the rapidly dividing cells originated in the bone marrow and were precursors of the blood monocyte. The evidence is as follows: 1) Injection of immune lymphocytes into nonimmune, irradiated recipients failed to transfer positive skin reactions (4); 2) Injection of bone marrow cells from nonimmune donors to sensitized, irradiated animals resulted in positive responses (5); 3) Local injection of nonimmune macrophages into the skin of irradiated, sensitized recipients elicited positive responses in these areas (6).

These studies indicated that two populations of cells are required to elicit delayed hypersensitivity.

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The first are radioresistant, which were later defined as long-lived, T lymphocytes. The second population are cells of the monocyte/macrophage system and are derived from radiosensitive precursors in the bone marrow.

From this information we can develop a concept of delayed hypersensitivity consisting of three components (Table 2). First, sensitized T lymphocytes, perhaps with some help from macrophages, recognize intradermally injected antigen. Secondly, antigenic contact triggers a response in sensitized lymphocytes. Within a few hours these cells release soluble mediators (7) called lymphokines into the environment and later, activated small lymphocytes transform into blasts and proliferate. Thirdly, through the amplifying effects of lymphokines, such as migration inhibitory factor (MIF) and monocyte chemotactic factor (MCF), other leukocytes, predominantly monocytes, accumulate at the skin test site and comprise the reaction of delayed hypersensitivity. This mononuclear infiltrate is similar to that of any other chronic inflammatory reaction. The triggering event, however, is an immunologically specific response of T lymphocytes.

Techniques are available to evaluate each of the "three R's." The number of circulating T cells available for antigen recognition can be quantitated by several methods. The most widely used method at this time is a rosette technique. When sheep erythrocytes are incubated with human lymphocytes at 4°C, the erythrocytes form rosettes around T cells (8). Secondly, heterologous anti-T lymphocyte serum has been developed (9) and may be used to quantitate T cells either by immunofluorescence or cytotoxicity.

The second component, response to antigen, can be evaluated by measuring the lymphocyte proliferative response to antigen. In these studies lymphocytes are cultured with test antigen for five-

TABLE 1
THYMUS-DERIVED LYMPHOCYTE FUNCTIONS

1. Delayed hypersensitivity
2. Activation of macrophages to resist infection
3. Rejection of allografts and tumors
4. Regulation of antibody production by B cells

to-seven days and proliferation is assayed by measuring incorporation of a radiolabeled DNA precursor.

Alternatively, one can measure lymphokine production from antigenically stimulated lymphocytes. Migration inhibitory factor has received the most attention (7). Various techniques to measure MIF have been developed which are based on the inhibition of leukocyte migration by the culture supernatants of stimulated lymphocytes. The method of Rocklin and David (10) correlates well with delayed hypersensitivity. These workers use guinea pig peritoneal macrophages as effector cells. Other workers have substituted human "buffy coat" leukocytes for guinea pig macrophages (11). Results obtained with these cells as the indicator cells, however, did not always correlate with cutaneous skin reactivity to antigen (12).

I prefer to measure MCF rather than MIF, because both *response* and *reaction* can be evaluated. With this technique we can detect mediator production from a given patient's lymphocytes and also evaluate the capacity of that subject's monocytes to respond to his own mediator. With a method to

measure leukocyte chemotaxis, we can evaluate both lymphocyte and monocyte function.

To perform this technique a chamber is divided into an upper and lower compartment by Nuclepore® membrane. Monocytes are placed in the upper chamber and the chemotactic stimulants in the lower chamber. The specific migration of cells from the upper to the lower surface of the membrane filter is quantitated (13).

These *in vitro* methods can be used to define the component that is defective in patients with impaired delayed hypersensitivity. Lymphocytes from children with a congenitally absent thymus gland (the DiGeorge syndrome and Nezelof syndrome) fail to form rosettes with sheep erythrocytes or to proliferate in response to mitogens. These children, then, do not have sufficient T lymphocytes capable of antigenic *recognition*.

Impaired cell-mediated immunity in chronic mucocutaneous candidiasis may be due to several defects, either in a *response* or *reaction*. Patients with this disease usually have adequate numbers of circulating lymphocytes but may have selective defects in lymphocyte or monocyte function. In some subjects, blast transformation is intact but MIF is absent (14). In others, mediator production is intact, but these patients' lymphocytes fail to proliferate in response to antigen (15). Finally, Snyderman (16) reported a patient with chronic mucocutaneous candidiasis whose monocytes responded poorly to a chemotactic stimulus. Although it is tempting to speculate that the defects detected with *in vitro*

TABLE 2
DELAYED HYPERSENSITIVITY
A COLLABORATIVE PARTICIPATION OF LYMPHOCYTES AND MACROPHAGES
IN AN IMMUNOLOGICALLY SPECIFIC INFLAMMATORY REACTION

Component	Assay System
1. The <i>recognition</i> by small (thymus-derived) lymphocytes of material which is antigenic to the host.	Quantitation of T lymphocytes by: <ol style="list-style-type: none"> 1. Spontaneous rosettes without sheep erythrocytes. 2. Immunofluorescence or cytotoxicity using heterologous antihuman thymocyte serum.
2. A <i>response</i> of sensitized lymphocytes as detected by the release of soluble factors (lymphokines) and later by cell division.	Generation of lymphokines <ol style="list-style-type: none"> 1. Migration inhibitory factor (MIF) 2. Macrophage chemotactic factor (MCF) Blast transformation and proliferation
3. A mononuclear inflammatory <i>reaction</i> , consisting predominantly of "activated" macrophages, which leads to the attack, destruction, and sequestration of autologous or foreign materials.	Monocyte response to MCF "Skin-window" assay

techniques accurately reflect an *in vivo* disturbance, the data must be interpreted with appropriate caution at this time.

Delayed hypersensitivity may be impaired in conditions without a demonstrable defect in cellular function. Serum factors inhibit lymphocyte function in certain diseases and several examples are listed in Table 3. The role of these serum inhibitors has not been defined. Moreover, primary disorders of lymphocyte function have not been differentiated from secondary effects caused by serum factor in many diseases with impaired cellular immunity. Recent studies from this laboratory suggest that anergy in such diverse diseases as systemic lupus erythematosus (SLE) and primary intracranial tumors (17) is caused by serum inhibitors rather than defective cells. Fractionation of inhibitory serum in patients with each of these diseases revealed an IgG factor that broadly suppresses reactivity of allogeneic as well as autologous lymphocytes. This inhibitory IgG factor has been named lymphocyte regulatory immunoglobulin (LRG) and increased LRG in SLE may, in part, explain decreased delayed hypersensitivity. Patients with SLE and other diseases also have a lymphocytotoxic antibody that may depress cell function.

In summary, I have attempted to develop a working model of delayed hypersensitivity that may be useful in understanding areas puzzling to the non-immunologist. The reader is urged to ask the following questions in order to evaluate scientific papers dealing with defective cellular immunity in various diseases. First, did the patients studied have adequate numbers of circulating lymphocytes and monocytes? Secondly, did the methods used evaluate a single component of delayed hypersensitivity or were several components analyzed? Finally, did the authors distinguish a primary cellular defect from secondary

humoral effects? Separation of delayed hypersensitivity into "three R's" may be helpful in organizing and understanding present information.

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TABLE 3

DISEASES WITH SERUM INHIBITORS OF LYMPHOCYTE FUNCTION

1. Hematologic and solid tumors
2. Systemic lupus erythematosus
3. Tuberculosis
4. Multiple sclerosis
5. Hepatitis
6. Lepromatous leprosy
7. Ataxia telangiectasia
8. Multiparous women
9. Multiple transfusions

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