Cells Involved in Cell-Mediated and Transplantation Immunity, II. A Consideration of the Functional Identity of the Cells Involved in Both Humoral and Cell-Mediated Immunity: A Phylogenetic Approach

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Abstract. The literature concerned with the types of cells that participate in the humoral and cell-mediated immune response has been reviewed. It is postulated that the initial cells that are involved in mediating both types of immunity are functionally identical in that both are antigen-reactive cells. In the case of the humoral immune response, the interaction of the antigen-reactive cells with the antigen leads to the release or transfer of "information" to the antibody-forming cell, resulting in the synthesis and secretion of antibody molecules. In the case of cell-mediated immunity, it is considered that the primitive antigen-reactive cell itself transforms into the sensitized cell which infiltrates the site of antigen administration.

A previous communication dealt with the relationship between the cells mediating the humoral immune response and those cells whose immunologic function is specifically neutralized in the induction of the state of immunologic tolerance.1 An attempt was made to unify these apparently diametrically opposed immunological states at the cellular level. Recent findings demonstrating the involvement of multiple cell types in the induction of the primary immune response in the mouse2-5 and the rabbit6 support the concept of multicellular pathways leading to the humoral immune response.7 These pathways may consist of one cell only—antibody-forming; two cells—antigen-reactive and antibody-forming; and three cells—processing (macrophage?), antigen-reactive, and antibody-forming.7 The nature of the antigen constitutes the determining factor as to the cell pathway it will activate. The antigen-reactive cell is morphologically identical to the antibody-forming cell,8 the lymphocyte nature of which has been well documented.9-11 However, these two cells have unique immunologic functions—the antigen-reactive cell possessing the capacity to recognize or cognize the native or processed antigen and releasing "information" which stimulates the antibody-forming cell to synthesize and secrete antibody. Immunologic tolerance has been shown to involve the inactivation or destruction of the specifically directed antigen-reactive cells with the result that no "information" is passed on to the antibody-forming cell.7,12

The aim of this report is to unify the fields of humoral and cell-mediated im-
munity insofar as the cell-types and intercellular reactions are concerned. No attempt will be made to delve into the actual intra-cellular events which govern these two types of immune states. A number of excellent monographs\textsuperscript{12,14} and review articles\textsuperscript{15–17} and a speculative but profound and provocative article by Humphrey\textsuperscript{18} have served to provide the author with the stimulus to present this concept.

In terms of the phylogeny of the immune response, the cell-mediated immune response would appear to be the more primitive as there are indications of an inflammatory-type resistance having evolved in the lower animal species, such as the invertebrates, in the absence of circulating antibodies which generally characterize immunity in the higher vertebrates.\textsuperscript{19–21} Cell-mediated immunity appears to be the immunity of choice with respect to the generally noninvasive organisms—those which tend to localize within a particular organ. A classification of such microorganisms would include the fungi, rickettsias, viruses, protozoa, helminths, and some bacteria. Under these circumstances, it would be economical for the host organism to create cells which would be specifically attracted to the focus of infection, to either liberate an agent specifically toxic toward the invading organism or physically interact with the latter and eventually wall off the infected area. It would be very extravagant on the part of the body to provide cells in a distant organ which would synthesize vast numbers of antibody molecules to combat a localized invader. In a review dealing with the immune mechanisms in helminth infections, Soulsby\textsuperscript{22} states that there is little evidence in nematode and trematode infections to show that circulating antibody is concerned in protective immunity. Furthermore, although little information exists with respect to the importance of white cells in the immune response, the fact that they occur in large numbers at the site of an immunologic reaction between the host and the parasite is probably not a fortuitous finding.\textsuperscript{22} On the other hand, a very large number of specifically sensitized cells would be required to combat a disseminated or generalized infection, especially when it is also accompanied by the secretion of exotoxins. To combat this type of infection, the host has evolved a specific defense mechanism based on the secretion of antibody molecules which circulate and meet the organism or toxin anywhere in the body and there neutralize it. This type of immune mechanism permits a conservation of the cell population with a concomitant lower expenditure of energy by the host since the energy expended to synthesize a large number of antibody molecules is of several orders of magnitude less than is necessary for the proliferation of a vast army of specifically sensitized cells. However, since immunity mediated by humoral antibody is dependent upon complement in order to successfully induce the lysis and death of the invading microorganism, it had to await the evolution of the complement system before it could become effective and displace cell-mediated immunity. It has been suggested that complement evolved among the lower vertebrates.\textsuperscript{23} It is therefore of more than academic interest to note that antibody synthesis appears to have evolved among the lower invertebrates. However, humoral immunity in the invertebrates and lower vertebrates is independent of the complement system and constitutes a modified or primitive cytotoxic system.\textsuperscript{19,20,23}
It has been the experience of a large number of investigators that the inhibition or delay of onset of the humoral immune response results in the unmasking and facilitation of the cell-mediated response. Thus, cell-mediated immunity rather than humoral immunity will result by varying the route of antigen administration, by subjecting the animal to a dose of total body irradiation capable of inhibiting antibody formation, or by administering a small amount of the antigen in the form of an antigen-antibody complex. In each of these cases, the humoral immune response is very much delayed in onset and is characterized by a low concentration of circulating antibodies. On the other hand, the induction of a state of cell-mediated immunity can be suppressed if the antigen is first administered in such a way as to evoke a humoral response. In fact, Crowle and Hu demonstrated that they could inhibit the occurrence of cellular immunity (delayed skin reaction) if they passively immunized the animal beforehand with homologous antiserum.

In retrospect, it might have been anticipated that the presence of circulating antibodies would normally prevent the manifestation of cell-mediated immunity directed toward the same antigen from being evident, probably as a result of neutralization of the antigen by circulating antibody before it can interact with the cell that mediates cellular immunity or the delayed hypersensitivity reaction. However, it could be postulated that these two types of immune mechanisms can only exist in series, and not in parallel, and that the cell(s) which would normally be stimulated to participate in the cell-mediated response could be diverted toward the synthesis of humoral antibody. That this is not the case was demonstrated by Crowle and Crowle, who observed that the lymphoid cells and the serum of a guinea pig immunized with ovalbumin could simultaneously transfer cell-mediated immunity (delayed hypersensitivity) and Arthus reactivity (humoral immunity), respectively, to normal recipients. More recently, Brostoff and Roitt demonstrated that humoral and cell-mediated immunity can coexist in the untreated grass-sensitive individual since the latter could give both a wheal and flare (immediate) skin reaction (humoral immunity) as well as a delayed skin reaction (cell-mediated immunity) if the former were inhibited by the administration of antihistaminics. Thus, it would appear that humoral and cell-mediated immunity are not mutually exclusive states induced by antigen stimulation.

In order to bridge the void that exists with respect to the cell types involved in manifesting humoral and cell-mediated immunity, it is suggested that the antigen-reactive cell constitutes the basic cell for both types of immune responses. This cell concerned with evoking humoral immunity is considered to be a more highly evolved cell of the short-lived variety of lymphocyte, and to inhabit a particular organ, such as the bone marrow or the thymus, rather than be in the circulation. The antigen-reactive cell concerned with evoking cell-mediated immunity may be more primitive with respect to antigen-reactive cell function, and it may be of the long-lived variety of lymphocyte. It is also present in the circulation of the normal animal since peripheral leucocytes can initiate a "graft-versus-host reaction" in the mixed leucocyte culture reaction. This consideration would permit an understanding of the radio-sensitive nature
of the humoral, but not of the cell-mediated, immune response, since a short-lived cell, because of its higher rate of metabolism and protein turnover, would be expected to be more susceptible to the deleterious effects of irradiation than a long-lived cell.

Assuming that the initial event in the induction of both types of immunity is antigen recognition, is the mechanism of recognition the same and what constitutes a recognition site on the antigen-reactive cell? Since the composition and nature of the recognition site is not known with respect to either of the two types of immunity, it might be expedient to consider that the recognition sites are essentially identical. This, in fact, has been suggested by Humphrey. In the case of humoral immunity, it is assumed that the recognition site on the virgin antigen-reactive cell consists of an antibody molecule or at least the antigen-combining fragment that is configurationally complementary to that of the antigen. Interaction of this site with the specific antigen stimulates the cell to undergo blastogenesis and mitosis, with accompanying synthesis of an excess of these “recognition sites” with their subsequent release from the cell, either free or combined with antigen (native or degraded) (Fig. 1). It is interesting that Paul Ehrlich postulated the release of similar antigen-reactive moieties from antibody-forming cells 70 years ago. This complex of recognition-

FIG. 1.—The relationship between humoral and cell mediated immunity.
site antigen may constitute the superantigen or immuno-carrier that has been
detected in the circulation of the immunized animal.45,46 This complex would
possess a high affinity for the antibody-forming cell with which it would interact
and trigger off the proliferative and transformational events culminating in
humoral antibody formation. On the other hand, it is postulated that the more
primitive cell-mediated immunity has not evolved as far as the antibody-forming
site to an appreciable degree after the interaction with the antigen. Rather,
this cell transforms into the sensitized cell. However, the "recognition
sites" that were liberated as a result of cell death or other cause are capable of
passively sensitizing immunologically uncommitted lymphoid cells (Fig. 1).
The findings of Freedman et al.47 and Perey et al.48 support this interpretation.
Freedman et al. observed that cell-free extracts of peripheral leucocytes obtained
from PPD-negative and positive individuals could agglutinate red cells sensitized
with PPD, although the titers of extracts of cells of PPD-negative individuals
were uniformly lower than those of cells from PPD-positive individuals. How-
ever, since the sera of these individuals also possessed antibodies in high titer to
PPD, it may be that Freedman et al. detected cytophilic antibody. Furthermore,
the fact that tuberculin-negative individuals possessed antibodies to PPD is
generally considered to represent the consequence of subclinical immunization
with tuberculin. Perey et al.,46 in a recent communication, reported that they
could passively transfer skin homograft immunity in chickens with plasma ob-
tained from sensitized x-irradiated bursectomized (agammaglobulinemic) donors.
They suggest that circulating antibodies do not appear to play a role in the
transfer of cell-mediated immunity and that the irradiated sensitized cells in the
irradiated donor probably released a substance(s) into the circulation that
could confer homograft immunity onto normal cells in a recipient animal. They
consider that this substance (recognition sites?) is quickly removed by receptor
sites on other lymphoid cells but they did not investigate how long this substance
could be detected in the circulation following x-irradiation.

Although Nelson and Boyd6 claim that cytophilic antibody is not involved
in the mediation of the delayed hypersensitivity reaction, Amos et al.50 have
demonstrated that guinea pig gamma 2 cytophilic antibodies can effect inhibition
of macrophages in the presence of the specific antigen. It is generally agreed15
that the two biologically active agents concerned—transfer factor and migration
inhibitory factor—are not related substances and that the migration inhibitory
factor is released as a result of the interaction between the sensitized cell (transfer
factor?) and the antigen. The migration inhibitory factor then acts on normal
macrophages to immobilize them. Whether this factor acts in an immunolog-
ically specific or nonspecific fashion cannot be ascertained at the present time,
although Amos and Lachmann51 have recently demonstrated that antigen is
required for the inhibition of migration of normal macrophages by cell-free
supernatants (migration inhibitory factor?) obtained from cultures of sensitized
guinea pig lymph node cells and antigen.

Evidence in favor of the functional and biological, if not chemical, identity
of the recognition sites on the antigen-reactive cell which mediates humoral immunity and the sensitized cell which mediates cellular immunity emanates from recent observations on the migration capacities of lymphoid cells in vitro in the presence of antigen. A large number of investigators have demonstrated that interaction of peritoneal exudate cells of sensitized (i.e., old tuberculin) animals with the antigen in vitro results in inhibition of migration of these cells.\textsuperscript{15,52–54} Falk and Falk\textsuperscript{55} have observed that the migration of normal rat thymus cells in vitro can be inhibited by a number of antigens of divergent composition, although the cells of the other lymphoid organs were not so affected. Preliminary observations with the rabbit in this laboratory suggest that the in vitro migration of normal rabbit bone marrow cells can also be inhibited by antigens.\textsuperscript{56} It is interesting to note that the prime organ sources of the antigen-reactive cell appear to be the thymus in the mouse\textsuperscript{2–5} and the bone marrow in the rabbit.\textsuperscript{40,42,43,57,58} These findings strongly suggest that the antigen-reactive cell that mediates the humoral immune response can interact with the antigen in vitro as a result of which their migration, and that of the other bystander cells, is impeded or inhibited. \textsuperscript{59} Falk has also observed that the in vitro migration of normal human thymus cells obtained from a tuberculin-negative individual can be inhibited by incubation with PPD, although thymus cells obtained from a tuberculin-positive individual could not be so affected by PPD in vitro, which suggests that the thymus may indeed be the source of virgin antigen-reactive cell in man. This finding suggests that the human thymic antigen-reactive cell vacates the thymus, just as that cell in the rabbit vacates the bone marrow after antigenic stimulation.\textsuperscript{7,40} It would therefore appear that the recognition sites on the surface of the virgin precommitted antigen-reactive cell mediating the humoral immune response impart to the cells the property which permits their reaction with the antigen to manifest itself in a manner indistinguishable from that of the interaction of the antigen with the "sensitized cell" participating in cell-mediated immunity, that is, inhibition of cell migration in vitro.

It is interesting to note that only 1–4\% of the cells infiltrating a site of a delayed hypersensitivity reaction have been shown to be sensitized cells.\textsuperscript{60–62} Since most of the infiltrating cells were not originally sensitized to react with the antigen, they therefore must have been passively sensitized as a result of interaction with a factor released by the sensitized cell (migration inhibitory factor?) which would render them capable of interacting with the antigen and of being immobilized at the site of injection of the antigen (Fig. 1). Falk et al.\textsuperscript{63} arrived at a similar conclusion on the basis of results obtained with normal and sensitized rat spleen cells in vitro. Only supernatants of cultures of sensitized cells with the antigen (sheep red cells or allogeneic spleen cells) could inhibit the migration of syngeneic cells in vitro. Since the inhibition of migration affects the whole cell population, they concluded that an active substance is released from sensitized lymphocytes incubated with the antigen which can then react with "innocent bystander cells" in such a way as to prevent their migration.\textsuperscript{63} Such a mechanism would provide an explanation for an apparently insoluble dilemma—how to account for the large percentage of cells capable of participating in the cell-mediated reaction. Although the clonal selection theory would not permit more than $1/10^4$–$10^5$ antigen-reactive cells mediating the humoral immune
response to be specifically precommitted with respect to any one antigen,\textsuperscript{12,64–66} it has nevertheless been observed that as many as 1–2% of virgin cells are capable of mediating cellular immunity.\textsuperscript{67,68}

Additional evidence suggesting the functional identity of the cells mediating humoral and cellular immunity derives from studies on the blastogenic capacities of lymphoid cells. It is generally agreed that a state of delayed hypersensitivity must exist in order for sensitized or immune cells to undergo blastogenesis and mitosis in the presence of the antigen.\textsuperscript{39,69–71} Furthermore, normal rabbit bone marrow lymphocytes\textsuperscript{42,43} and normal mouse thymic cells\textsuperscript{72} are also capable of undergoing blastogenesis upon stimulation with antigens. Since the normal cells exhibiting this activity are considered to be virgin, precommitted antigen-reactive cells, it would appear that the normal antigen-reactive cells mediating humoral immunity cannot be distinguished from the sensitized cell mediating cellular immunity on the basis of the blastogenic response to antigen.

One may indeed speculate as to whether the “potentiating factor” released from cultures of normal cells, recently described by Janis and Bach,\textsuperscript{73} might not in fact be “recognition sites” capable of passively sensitizing other uncommitted lymphoid cells. This substance, released by peripheral blood lymphocytes in culture, is capable of potentiating the blastogenic effect of antigens (i.e., PPD) \textit{in vitro}.

Although it has been demonstrated that the antigen-recognition site on an \textit{immune} cell is antigenically an immunoglobulin molecule or fragment,\textsuperscript{74} no evidence has as yet been presented to suggest that the recognition site on the \textit{virgin} immunocompetent cells is protein in nature. Lawrence has presented evidence\textsuperscript{15} that “transfer factor” mediating cellular immunity is probably a polynucleotide. If “transfer factor” is not any antibody molecule but rather a “recognition site” released from the virgin antigen-reactive cells mediating cellular immunity (Fig. 1), then might not the recognition site on the surface of these cells mediating humoral immunity possess the same composition? The polynucleotides may, in fact, represent a specifically coded sequence of nucleotides, capable of reacting with antigen. The antigen-reactive cell would then release the complex composed of recognition site and antigen which would be accessible to the antibody-forming cell and would transfer specific information to the antibody-synthesizing apparatus of the antibody-forming cell. This information would be translated by the latter cells into the amino acid sequence of the antibody-reactive site on the antibody molecule which would be configurationally complimentary to the antigenic site on the antigen molecule. This information, in the form of specific polynucleotides, would cause an alteration in the basic ribosomal RNA template followed by a corresponding alteration in the synthesis of antibody immunoglobulins. Such a mechanism would provide for the recruitment of uncommitted antibody-forming cell when the situation would require it. The chromosomal DNA would not be affected at all and therefore it would not be necessary to postulate the transmission of immunologic information to progeny cells. Thus, this concept would conform with the template theory of antibody formation postulated by Breinl and Haurowitz 40 years ago\textsuperscript{75} and subsequently modified by Haurowitz.\textsuperscript{76}

The objective of this communication was to unify the fields of humoral and
cell-mediated immunity. Evidence has been presented to support the postulated scheme. As the cells and mechanisms presented above are amenable to laboratory investigation and verification, it should be possible to determine the validity of the proposed mechanisms within a relatively short period of time. In any case, this presentation should provide a stimulus for a more penetrating and analytical approach toward the elucidation of the cells and cell-interactions concerned with the humoral and cell-mediated immune responses.

Abbreviation: PPD, purified protein derivative from Mycobacterium tuberculosis.

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48 Likhite, V., and M. Richter, unpublished results.
53 Likhite, V., and M. Richter, unpublished results.
56 Balk, R. E., personal communication.