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Is There an Immunologically Incompetent Lymphocyte?

By John G. Gorman and James G. Chandler

Is there an immunologically incompetent cell? Much evidence suggests that there is.

In 1938 it was argued that a completely hypothetical particle called the neutrino must exist if certain physical phenomena were to be explained. The necessary properties of this particle such as mass, charge, energy, etc. were predicted. Only recently experiments designed to "catch" a particle with these properties were successful.1 In this essay it will be argued from purely theoretical considerations that the "immunologically incompetent" (I.I.) cell, an immunologic cell type with a hitherto unrecognized function, must exist if certain immunologic phenomena are to be explained. The necessary properties and function of this postulated cell will be defined. What is this concept? The competition theory2,3 proposes that immunologic tolerance is a proliferative cellular response to antigenic challenge rather than, as all other theories hold, a specific lack or inhibition of cellular proliferation. Tolerance is therefore another kind of immune response exactly analogous to the proliferation of specific antibody-forming plasma cells or other immunocompetent4 (I.C.) cell types that follow injection of antigen. However, the theory envisages a class of "immunologically incompetent" cells which multiply in response to specific antigen stimulus and produce tolerance because of their failure to manufacture antibody or mediate any other destructive effect on their specific antigen. However, these cells are complete competitors of immunocompetent (I.C.) cells responding to the same antigen and therefore able to prevent the proliferation of any one of the destructive types of immunocytes4 with specificity for the same antigen by a process of competitive exclusion.5 Partial tolerance6 occurs when an antigen induces a mixture of I.I. cells and conventional I.C. cells, i.e., a mosaic clone.

Let us review the features of acquired immunologic tolerance. Tolerance is usually induced by what amounts to a course of immunization with antigen.6,7 Its specificity is exactly like that of immunity8 and like immunity, tolerance has the property of cross-reactivity in that an individual made tolerant of one antigen has some degree of tolerance to antigens with similar molecular configuration.8 Tolerance can exist at all levels of intensity—that is, all degrees of partial tolerance may be seen.6 Moreover, booster injections of antigen are required if tolerance is to be maintained at a high level, just as with immunity.7 Dresser has shown that the level of tolerance developed

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Fig. 1.—Origin of an immunologic clone. Under antigenic stimulus (f) a stem cell becomes committed to the manufacture of anti-f antibody and then multiplies to form a clone of cells all manufacturing anti-f antibody only. While small, this clone grows exponentially because there is plenty of space for it, but finally reaches a size limit or equilibrium defined by cell homeostatic mechanisms.

In adult mice after a single tolerance inducing injection of BCG increases with time. No tolerance existed 1 hour after antigen challenge, increasing tolerance was seen at 3, 5 and 8 days and all animals were completely tolerant at 12 days after injection of antigen. The time relationships of the development of tolerance, therefore, are surprisingly similar to those of the conventional immune response. All of these similarities with immunity, not often emphasized, are expected by the competition theory which holds that tolerance is due to proliferation of a special class of immunocytes following antigenic challenge.

When this theory is applied to the phenomenon of tolerance to self, some interesting implications emerge. To ensure tolerance to self, each of the thousands of self-antigens must induce in fetal life a clone of specific II. cells whose direct descendants persist, stimulated continuously by self-antigen,
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Fig. 2.—Origin of a mosaic clone. An antigen may sensitize more than one stem cell and start two clones both having the same specificity. At first, both can grow exponentially but later growth is slowed because of competition for space since both clones are subject to the same cell limit control mechanism. In this paper the term clone refers to all immunologic cells specific for one antigen and therefore it may be a “mosaic” composed of more than one type of immunologic cell.

throughout the entire lifetime of the individual. Because each of the thousands of self-antigens must have its clone, the theory requires the existence in the lymphoid system of enormous numbers of this special cell type. The theory would fail right here if such enormous numbers of lymphoid cells, having a morphology at least not inconsistent with an immunologically incompetent function yet remaining uncommitted to any other obvious and essential function, could not be found. Fortunately the theory passes this first crucial test and indeed yields a fine dividend. The small mature lymphocyte, the only cell present in the numbers necessary for this I.I. function, is given an essential purpose for its existence and its special morphology.\textsuperscript{11}

Autoimmunity occurs when I.C. cells for any reason begin to displace I.I. cells from the clones against self-antigens. Note that in this model autoimmunity is equivalent to partial tolerance to a self-antigen, i.e., the development of a mosaic clone of I.C. as well as I.I. cells directed against a self-
Fig. 3.—New model of the lymphoid organ. The lymphoid organ is under homeostatic cell controls that limit its total size. Clones competing for space in this large niche have limits also. Each self-antigen is stimulating a clone filled to the limit with immunologically incompetent cells and this ensures tolerance to self. Foreign antigens have clones filled with immunologically competent cells which manufacture antibody.

antigen. Oscillations in the ratio of these two competing populations are responsible for exacerbations and remissions which are such a common feature of autoimmunity.

Before some of the instances are cited where the concept has real advantages over other theories, some general ecological principles will be emphasized which must be grasped and applied before the full usefulness of this theory can be appreciated. The dynamics of lymphoid cell populations are no different from those of populations of any other kind. The principles of ecology apply to lymphoid cells, just as they do to any other body cells or to other populations, whether they be animal, plant or bacterial. Any biological population will always increase in number until it completely fills an ecological niche which means that it reaches a maximum limited by space or food supply or is stopped by some more subtle homeostatic control mechanism. Two populations which have similar origins, structures, functions and sites of activity compete for space in the same ecological niche. The competitive exclusion principle states that in this situation it is usual that one population will completely displace the other from the niche. This competition is of course identical with the concept of Darwinian selection or survival of the fittest.5

The cells of the lymphoid organ, although scattered throughout the body, must constitute a population occupying an ecological niche, the lymphon, whose total cell number is under biological control just like the red cell mass, another scattered cell population with limitations on total numbers. According to the competition theory the lymphon must be pictured as the total of many individual clones, each specifically directed against an antigenic determinant, either self or foreign present in the body and each competing with the others for ecological space. Some hint of the dynamics of this competition in the whole lymphon, or what will be called the “large niche,” can be obtained by consideration of the following experimental observations. Competition of antigens is a well known phenomenon explained by the fact that rapid expansion of a clone responding to one antigen may preempt space needed by cells responding to a second antigen simultaneously.12 A lymphoma, which is an
enormous monoclonal proliferation of lymphoid cells, takes up so much of the ecological space of the lymphon that little is left for the normally functioning cells such as the clones of immunocytes responding to foreign antigens. The expected failure of antibody production, absence of plasma cells, hypogammaglobulinemia and failure of delayed hypersensitivity and homograft rejection are well documented in cases of lymphoma. When the lymphocyte populations are depleted by whole body irradiation, prolonged drainage of lymphocytes into the esophagus from the thoracic duct, or by thymectomy in the newborn period, there is an enormous compensatory proliferation of plasma cells to fill the emptied niche with an alternate type of immunocyte. Following splenectomy there is a marked compensatory increase in the number of lymphocytes in the bone marrow. Infusion of enormous numbers of isologous lymphocytes will suppress the growth of a lymphoma in mice.

A distinction must be made between “clonal selection,” a theory of immune tolerance based on deletion of specific stem cells, and the “immunological clone,” a concept of the basic mechanism of formation of the immune response. What is the clone concept? It means that the effect of antigen when injected is first to select or instruct a relatively small number of immunologic stem cells so that they become committed to the particular antigen. Under continued antigenic stimulus these few specifically committed immunocytes multiply rapidly, at the same time each cell becomes more differentiated. The large number or clone of immunocytes seen several days later are all direct descendants of that small band of cells originally committed by the antigen. In the growth or formative phase of a clone (of plasma cells for example), the number of antibody synthesizing units and the rate of antibody production per cell is rapidly increasing, apparently exponentially. The clone concept specifies that all immunocompetent cells that appear after antigen injection are newly grown cells and denies that large numbers of differentiated cells already manufacturing one antibody convert their protein synthesizing apparatus so as to be able to manufacture antibody of new specificity. This point, expected to be true for theoretical reasons, has been proved experimentally by Nossal and Makela who showed that all cells manufacturing antibody had incorporated tritiated thymidine and therefore were newly formed by mitotic division since the antigen stimulus. Gowans has shown by similar labelling that all the lymphocytes invading a graft rejection site are all newly formed cells.

When a population has to arise by multiplication from small numbers it will have a characteristic logarithmic growth curve. There is also an unavoidable time delay in reaching full size, dependant primarily on the generation time of the replicating units. The time delay between antigenic challenge and immune response is probably one of the most fundamental characteristics of immunologic responses and of course their logarithmic character has been often stressed.

The logarithmic growth phase of a clone does not last more than 10–14 days in a primary response and less in a secondary response. At this time,
the clone appears to reach a limiting size and, despite further antigenic stimulus, will not grow any larger. Obviously there is a homeostatic cell control mechanism operating which limits the number of immunocytes which any one antigen can command in its immune response. The exact mechanism of this control is unknown but that it must exist is self-evident. In ecological terms the clone has grown until it has filled its specific small niche and no further growth can occur. There are several alternative types of immunocyte which can multiply to fill the ecological niche created by injection of a new antigen, and whether specific gamma_1 M, gamma_1 A or gamma_2 globulin-producing plasma cells, delayed hypersensitivity lymphocytes, homograft-rejecting lymphocytes or I.I. lymphocytes or mixtures of these multiply to fill the niche will depend on many factors.

Once the clone has reached its maximum size a steady state exists in which, with continuing antigenic stimulus, the population replaces itself by mitotic division at a rate exactly equalling the loss of old mature cells. This population of immunocytes committed to, stimulated by and specific for the particular antigen can be thought of as a distinct population of cells occupying a controlled “small ecological niche” commanded by the antigen.

The immunologic status of the individual with regard to that particular antigen will be governed by the type of immunocytes occupying the niche and their relative proportions. The types and proportions of immunocytes persisting long after antigen stimulus constitutes the basis for “immunological memory.” It is evident that a continual state of competition must exist between the various cell types, that changes in the composition of the population may occur and that one cell type might in time emerge as the dominant cell type of the “niche.” Various manipulations such as altering the dose or route of injection of antigen, the use of adjuvants, x-ray or 6-MP or other chemotherapeutic agents could have the effect of giving one type of immunocyte a selective advantage over another and cause a changeover in composition of immunocytic types in the “small niche.” Such cell type changeovers would be reflected as a change in the individual’s immunologic status with regard to the antigen concerned. Examples are a change from 19S to 7S antibody of the same specificity, or from a state of delayed hypersensitivity to antibody type of immunity, or from tolerance to immunity as occurs following whole body irradiation. If 6-MP acts by favoring I.I. cells over I.C. cells, then it should produce its most dramatic effect when given early in the formation of the clone when it could influence what type of immunocyte fills the niche in the first place.

One good example of competition within the small niche is the often noted changeover of saline-Rh antibodies to albumin-Rh antibodies. Saline anti-Rh antibodies are a 19S gamma_M macroglobin, and albumin-Rh antibodies are 7S gamma_2 globulins. Mellors has shown that these two classes of immunoglobulin are manufactured by separate populations of plasma or “plasma-like” cells in lymph nodes from patients with rheumatoid arthritis. Changeover of Rh antibody type must represent a competitive exclusion of a 19S anti-Rh clone by a 7S clone from the ecological niche committed to the Rh antigen.
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Fig. 4.—Schematic illustration of reversal of autoimmune process by 6-MP. The "small niche" is depicted on the right with its control limit as in earlier diagrams. On the left is illustrated how the cellular composition of a "small niche" may change in time due to a differential in rate of growth of constituent populations. The application of this concept to remission of autoimmunity induced by 6-MP is depicted here. The persisting ratio of various immunocytic types is the basis of "immunological memory" which may also be manipulated by 6-MP.

Historically, the saline test, the only test for-Rh antibody available between 1941 and 1944, could detect the 19S-Rh antibody but not the 7S-Rh antibody. It was noted that the 19S antibody would often disappear in some individuals on antigenic stimulation. Had the concept then existed, this would have seemed like tolerance to early investigators because they could not detect the albumin antibody which was taking the place of the saline. In the same way, what seems like tolerance to us may be the displacement of specific immunocompetent cells by an alternate cell type which our present methodology cannot detect. In order to accept the postulated role of I.I. cells in producing tolerance, the only assumption necessary is that the clone control mechanism, rather than differentiating between the various types of immunocytes, merely controls the total of all types responding to a particular antigen.

Thus far, an attempt has been made to describe an ecological approach to the dynamics of lymphoid populations without referring to many specific situations. Let us now examine several specific observations selected because they are readily explained by the present model but pose difficulties for other theories.

a. A partially tolerant animal has a specific impairment of its ability to produce full levels of antibody although it may produce enough antibody to
more sensitive lymphocytes depleted leaving plasma cells

more plasma cells means worse autoimmune disease

Fig. 5.—Worsening of autoimmune disease by x-ray. Theory predicts that autoimmune disease may be dramatically worsened by total body x-ray because with the removal of the specific I.I. cells by x-ray, space is created for the corresponding I.C. cells to proliferate in their place. That x-ray can destroy partial tolerance can be explained by exactly the same mechanism.

indicate that the I.C. clone responding to antigen has reached a considerable size. This situation is readily explained under the competition theory by postulating a mosaic clone or a mixture of I.C. and I.I. cells in the cellular response to antigen. On the other hand, clonal selection23 and stem cell theories28,29 of tolerance encounter real difficulties here because they explain limitation of clone size by a limitation of the number of available stem cells capable of responding to antigen. Since, with logarithmic growth, even a single stem cell responding to antigen is potentially capable of growing into a large clone, it is impossible to achieve control of clone size merely by rationing stem cells, as clonal selection and stem cell theories propose. This is like expecting that the final size of a small pox epidemic can be accurately controlled by regulating the number of cases introduced into a country with a susceptible population. One case may be too many and once an epidemic is established its final size will depend on the effectiveness of measures to detect and isolate cases, of contact vaccination and mass vaccination programs and will be independent of the original number of cases that started the epidemic. It is obvious that the control of I.C. cell numbers must be exerted after the clone
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is established. “Nature abhors a vacuum” and unless competing I.I. cells were present there would be nothing to prevent an I.C. cell clone from multiplying to its full size once it was established. Consideration of states of partial tolerance, and of very mild autoimmunity and its remissions, reveals the fatal logical flaw in clonal selection and stem cell theories. They completely fail to provide a mechanism for controlling I.C. clones at reduced numbers. On the other hand, variable degrees of limited immunity to self or foreign antigens are very naturally explained by the mosaic clone concept.

b. Experiments performed to abolish tolerance by adoptive transfer of isologous I.C. cells also suggest the presence of a competing I.I. cell population. Adoptive transfer of 5 x 10⁶ isologous spleen cells from a normal or even an immune mouse completely fails to affect the tolerant state. Such a number of cells is known to contain sufficient numbers of I.C. stem cells to mount an effective immune response and could thus be expected to repair a “central” failure of the immune response. Actually, enormous numbers of cells of the order of 500 x 10⁶ cells must be transferred to abolish tolerance effectively.

c. Battisto has recently shown that a small dose of antigen injected into the portal vein will induce tolerance whereas the same dose injected into the jugular vein induces immunity in adult guinea pigs. It is more likely that this manipulation caused a selection of an I.I. cell response instead of an I.C. cell response rather than that the antigen was able to delete all the I.C. stem cells. The latter are known to be widespread in spleen and lymph nodes (from cell incubation and transfer experiments) and should not be more vulnerable because of an unusual site of injection.

d. Stem cell theories do not require the very large numbers of small lymphocytes that do exist, since clones can arise from very few stem cells. Recent work showing that small lymphocytes can, under rather unnatural conditions, change into large, rapidly dividing pyroninophilic cells does not affect the present argument. It is clear that the vast majority of small lymphocytes retain their ordinary morphology and do not divide throughout their relatively long lifetimes. Presumably, once an I.I. cell adopts its I.I. function and morphology it remains committed to this function under normal conditions.

e. The obliteration of tolerance (partial) by whole body irradiation is readily explained by postulating that I.I. cells are thereby deleted from the small niche, allowing rapid proliferation of the more radio-resistant I.C. cells remaining. The same explanation fits equally well the following observation of Schwartz. A percentage of F₁ mice injected with parental cells become chimeras but do not get runt disease. Presumably the graft cells have become at least partially tolerant of host antigens. Following whole body irradiation a severe runting syndrome of rapid onset develops in these mice.

f. In lymphoma, myeloma or macroglobulinemia, the lymphoid cell tumor is expected to displace normal I.I. cell populations as well as I.C. populations. Since I.I. cells are the postulated mechanism of tolerance, autoimmune processes would be expected in conditions where I.I. cells are displaced, as in lymphoma. This association is now well documented. In addition, following treatment of the lymphoma with x-ray or drugs, a vast amount of ecological space would be made available for lymphoid cells. I.C. cells might find
If Lymphoma is destroyed by therapy

Repopulation may result

In mosaic auto-immune clones.

Fig. 6.—Effect of therapy of lymphoma. Overgrowth of a clone to form a neoplasm—lymphoma, myeloma or macroglobulinemia—produces two effects because it occupies space in the lymphon. (1) Suppression of antibody formation against foreign antigens (e.g., (2) Interference with I.I. clones against self resulting in autoimmunity. Effect (2) is greatly enhanced by tumor destruction making space available for I.C. cells to grow during the ensuing clonal repopulation.

space to proliferate in the small niches against self-antigens. This would mean a dramatic onset or worsening of autoimmune phenomena following therapy. Many instances of this phenomenon with exactly the expected time relationships have now been recorded.

g. Zaalberg et al. injected parental bone marrow and lymphatic cells into lethally irradiated F, mice. These mice were shown to be chimeras entirely populated by parental lymphoid cells. Selecting as secondary donors those mice which did not get runt disease—that is, those in which the donor cells had become tolerant of F, antigens—they transferred bone marrow to one group and bone marrow and lymph node cells into a second group of lethally irradiated mice of the original parental strain. Those mice receiving bone marrow alone rejected skin grafts of the second parental strain of the F, combination but those who received lymph node cells as well were found to be tolerant of such grafts. The tolerant lymph node cells were therefore able to transfer specific tolerance to an animal that would otherwise be immunologically reactive. Stem cell and clonal selection theories cannot explain this result.
The concept of the I.I cell and of clonal competition have been extensively tested for their ability to explain immunologic phenomena described in the literature. Although the I.I. cell is still a completely hypothetical concept, it can greatly simplify many of the complicated and unlikely explanations needed by other theories of tolerance. So far, no evidence has been found in the literature nor has any been adduced in discussions with many immunologists, which is fatal to the I.I. cell concept. It is hoped that the cogent theoretical reasons for believing in the existence of the I.I. cells will stimulate the development of methods capable of directly detecting the activity of such cells.

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