HOW T LYMPHOCYTES RECOGNIZE ANTIGEN

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I. INTRODUCTION

Before the delineation of the two broad classes of immunocompetent lymphocytes, known as T cells and B cells, one of the paradoxes of immunology was known as the "carrier effect". In essence, the carrier effect was the failure of animals immunized with a simple hapten to produce anamnaestic antibody responses, specific for the hapten, upon boosting with that hapten coupled to any protein carrier other than that used in the primary immunization. The real difficulty that immunologists had in dealing with this observation was that the antibody produced reacted equally well with the hapten on any carrier. Thus, while the product of the response was hapten-specific, the responsiveness of the system itself was not. Hence, the simple model which stated that lymphocytes had receptors that were antibodies was called into question.

A partial answer to this problem came when it was discovered that there were two synergistically acting classes of lymphocytes involved in antibody responses. One class (B cells, since in chickens they are derived from the Bursa of Fabricius) carries as its receptor an antibody molecule identical in its antigen-binding characteristics to the antibody it will produce upon stimulation with antigen. The other lymphocyte, called a T cell (since it matures in the thymus), was required for the response of the B cell and showed specificity for the carrier.

One of the most intriguing problems in immunology during the years since the discovery of T cells as an entity has been the nature and specificity of their receptors. The study of the antigen-binding receptors in T cells has been plagued by a number of technical difficulties that are just now being solved. Many puzzling properties of T cell responses to antigen, including the failure of T cells to make hapten-specific responses in most cases, still remain to be elucidated. In this chapter, we will first consider a number of these puzzling features of T cell specificity. We will then consider some recent advances in understanding T cells and their receptors which may help to explain these results. Finally, we shall try to explain findings about T cell functional specificity in the light of this recent information.

Most information about the specificity of T cells has come from studies of their functional responses to antigen in complex experimental situations. These studies have by and large been carried out using either unfractionated lymphocytes or preparations depleted of B lymphocytes (T cells). One essential concept that has emerged over the past several years is that T cells are comprised of numerous subpopulations. Furthermore, recent evidence suggests that cells in one subpopulation (or set) may have receptors that differ from those of cells in another set. Thus, the study of T cell receptors may have to be redefined, since not all T cells may have qualitatively similar receptors. We will describe the current state of knowledge about T lymphocyte sets and their

specific functions, and illustrate this with experiments that suggest that such cells have differing receptor specificities.

A second group of findings that has profound implications for the nature of T cell receptors, and antigen recognition by T cells, has been the discovery that T cells do not in general recognize antigen per se, but rather recognize antigen in the context of self structures. Thus far, the predominant self structures involved in these processes are encoded in the animal's major histocompatibility complex (MHC). Indeed, this finding has opened the way to an entirely new interpretation of the function and nature of immune response (Ir) genes, which are encoded within the MHC.

Finally, immunoglobulin genes play a clearly demonstrated role in T cell specificity, namely in encoding the antigen binding portion of T cell receptors. While much of the evidence for this conclusion is still indirect, new information continually strengthens the contention that T cells employ conventional variable region genes from the Ig-H chain locus (VH genes) to make antigen-binding receptors. Furthermore, recent evidence suggests very strongly that T cells use a more limited repertoire of VH genes to make their receptors than is found on B cells or circulating Ig. The reasons for this are not apparent, but the net result may be to give rise to unexpected specificity patterns in the responses of such cells.

When the findings in these three areas have been considered in detail, we shall attempt to explain various puzzling functional specificities of T cells in the light of these recent data.

II. ANTIGEN RECOGNITION BY T CELLS

Antigen recognition by T cells has been studied primarily by means of functional assays of T cell responsiveness. While B cells make large amounts of a product (antibody) that is identical in binding specificity to the antigen-binding receptor they carry, isolation of T cell receptors in a form in which they can bind antigen has only recently been achieved. Data from these studies will be considered later in this chapter. The purpose of this section is not to exhaustively review data on specific T cell responses to antigen, but rather to describe certain puzzling aspects of such studies that may serve to illustrate the functional specificity of these cells.

Studies of T cell specificity have generally employed either hapten-protein conjugates or families of evolutionarily related proteins such as mammalian serum albumins. An animal is immunized to one of these antigens, and responses to it and to other members of the family are measured in some functional assay. The antigens used in these studies will be referred to as "nominal antigen", a useful term introduced by Thomas and Shevach.[1] This term is used because the actual antigenic complex recognized appears to involve self structures as well (see Section IV). In considering these responses, it is also important to remember that T cell responses almost always involve more than one cell type responding to antigen, a subject that will be explored in more detail in Section III.

In the introduction, the carrier effect was described, and it was pointed out that hapten-specific T cells do not generally result from immunization with hapten-protein conjugates. However, a number of workers have succeeded in producing populations of T cells that will respond to haptens on carriers other than those used for the immunization. In examining these apparently exceptional instances, the nature of the carrier molecules employed may tell us something important about the specificity of T cell responses to antigen. It is also necessary to point out that true hapten specificity has not been definitively demonstrated in any of these cases, since in no instance has free hapten interfered with antigen recognition by the T cell. However, responsiveness to a given hapten on a wide variety of carrier molecules is both interesting and informative.

TABLE 1

Hapten Recognition by T Cells and Antibody

Hapten-carrier conjugate	T cell response	Binding to anti-DNP-antibody
2,4-Dinitrophenyl-protein	+ + + +	+ + +
Ortho-nitrophenyl-protein	0	0
Paranitrophenyl-protein	+ + +	+ + +
2,6-Dinitrophenyl-protein	±	±
2,4,6-Trinitrophenyl-protein	+ + +	+ + +
2,4-Dinitrophenyl-β-alanyl-glycyl-glycyl-protein	0	+ + + +
2,4-Dinitrophenyl-lysine	0	+ + +

Note: The responses of T cells from guinea pigs immunized with 2,4-dinitrophenyl-mycobacteria to various hapten-conjugates, and binding of antibody from the same animal to the same hapten-conjugates.

After Janeway, C. A., Jr., *Transplant. Rev.*, 29, 164, 1976. With permission.

The first example of hapten-specific T cell responsiveness was the finding of Leskowitz and Jones[2] that animals immunized with the low molecular weight antigen arsanilazo-tyrosine (ABA-Tyr) in complete Freund's adjuvant would reproducibly induce delayed-type hypersensitivity in guinea pigs to the ABA hapten on any immunogenic carrier. Further studies on this system have not determined why this form of immunization should differ from immunization with ABA-proteins, which give rise to typical carrier-specific effects.

Subsequent studies by Alkan and el-Khateeb[3], Trefts and Henry,[4] and Janeway[5] have employed hapten coupled directly to *mycobacteria* to induce T cells reactive to a variety of different haptens on almost any carrier. These studies all agree in the basic finding that T cells immunized in this way will respond to the hapten coupled to any protein and that precise discrimination by T cells of different, but structurally related, haptens takes place. An example of this, including a comparison with the ability of serum antibody from the same animal to discriminate hapten is shown in Table 1. However, these studies are not consistent with the conclusion that the repertoire of specificities manifested by T cells is identical to that manifested by B cells. This is based on a number of findings.

The first of these is that, while DNP coupled to all naturally occurring proteins tested would induce proliferation in DNP-*mycobacteria* immunized T cells, DNP coupled to certain synthetic antigens would not induce such proliferation. This suggested that the hapten-reactive T cells were not fully hapten specific. This was confirmed in subsequent experiments in which T cells reacting to hapten coupled to one carrier were eliminated by the technique of 5-bromodeoxyuridine and light suicide (BUdR and light). The remaining cells would not respond to hapten on the original stimulating carrier, but would respond to the hapten on other carriers. While there was apparent overlap when natural proteins were used as carrier, there was always sizeable residual responsiveness to other hapten-protein conjugates. DNP-synthetic antigen stimulation gave even cleaner results.

TABLE 2

Cytotoxic T Cells can Discriminate DNP from TNP

	Average percent kill on autologous targets modified with	
Autologous modified stimulators	TNP	DNP
TNP	32%	6%
DNP	0%	11%

Data summarized from Forman, J., *J. Exp. Med.*, 146, 600, 1977. With permission.

A more decisive demonstration that T cells and B cells differed in their recognition of hapten was obtained using a tripeptide spacer molecule to couple the hapten to the carrier. In this case, the hapten coupled directly to the carrier would stimulate T cells, but the hapten coupled via the spacer would not (see Table 1). The same compounds, tested for their ability to stimulate B cells to produce antibody during adoptive secondary antibody responses, were found to stimulate B cells in the presence of T cells almost equally efficiently.[6,7] This result, while pleasing in its cleanness, does not yet have an adequate explanation in terms of T cell specificity. This finding is peculiar neither to the guinea pig nor to T cell proliferation assays, since the identical finding has been made by Rehn et al.[8] in a T cell cytotoxic system using the hapten 3-nitro-5-iodo-4 hydroxyphenacetyl (NIP).

Another interesting finding made using hapten-recognizing cytotoxic mouse T cells was that T cells could discriminate the hapten DNP from the closely related hapten 2,4,6-trinitrophenyl (TNP)[9] (Table 2). (However, this is not the case with hapten-*mycobacteria* immunized guinea pigs, Table 1.) It is interesting that Klinman and Press[10] have shown that DNP- and TNP-reactive unprimed B cells also comprise nonoverlapping sets of cells, despite the fact that their products crossreact extensively (90%) with the other hapten. Furthermore, Eisen and his co-workers[11] have made similar observations using antibody "quality" to discriminate anti-DNP from anti-TNP. Interestingly, both groups observed that B cells, once primed, do show considerable crossreactivity between DNP and TNP. These examples illustrate the necessity of studying the specificity of the B cells themselves, in addition to their products, before comparing them to T cell reactivity with the same antigen.

Because these T cell responses do show specificity not only for the hapten but also for the carrier and for the link between the hapten and the carrier, we prefer to call them hapten-reactive cells rather than hapten-specific cells. Furthermore, it would seem appropriate to reserve the term *hapten specific* to those instances in which the following criteria can be met (as they have been for B cells):

1. Cells react with the hapten on a variety of different carriers.
2. Carrier alone does not activate the cells.
3. A different hapten on the same carrier does not activate the cells.
4. Hapten coupled via spacer molecules to the carrier can also stimulate the cells.
5. Free hapten can block the response.

Despite the failure to show hapten-specific, or even hapten-reactive, T cells in the majority of experimental systems, there have been other instances in which hapten priming leads to the generation of T cells which will respond to the hapten coupled to

other proteins. In these instances, there appears to be a requirement for particular types of carrier molecules. These fall into three groups: autologous Ig,[12,13] lipid-rich carriers (such as *mycobacteria*) or lipid-substituted proteins,[4,5] and the response to ABA-Tyr (see above).[2] It is not clear at the present time why these compounds should be able to induce T cells to respond to hapten on other carriers. Undoubtedly, the answer to this question is relevant to the specificity of T cell responses to antigen. We will return to this question at the end of the chapter, when a tentative answer will be given.

One other characteristic property of T cells that has been brought out in a number of studies is their ability to respond to denatured antigens. This has been reviewed recently by Benacerraf,[15] and the evidence will be merely summarized here. T cells from animals primed with native protein will respond to the denatured form of the protein or to protein fragments, while B cells will not, nor will antibody bind to the fragments. This rather surprising behavior is again not explained at the present time. However, it is another indication that T cells may have a different receptor from B cells.

A property that certain T cell responses do apparently share with certain B cell responses is one termed *heteroclicity*. By this, we mean that the T cells bind or respond more strongly to an antigen different from that used for immunization than to the immunogen itself. Two recent examples of this are those presented by Krawinkel et al.[16] in which T cell receptors were isolated by binding to the hapten 3-nitro-4-hydroxyphenacetyl (NP) and were assessed for activity by their ability to neutralize haptenated phage particles. Inhibition of this reaction was then measured using the haptens NP and NIP. The latter were found to be significantly more potent, provided the T cells were derived from mouse strains of the Ig allogroup Ig^b, whose primary antibody response to NP shows the same binding pattern. These receptors will be further discussed below. The second instance also has been described in mice of the Ig^b type. It involves the T cell proliferative response to cytochrome c from various species. Mice immunized with pigeon cytochrome c respond much more strongly to tobacco horn worm cytochrome c than to pigeon cytochrome c, although the reason for this is not immediately apparent from the structure of the proteins themselves.[115] Heteroclitic antibody responses are associated with highly restricted variability in the antibody. The same is probably true of T cell responses. We will return to this at the end of the chapter.

Another puzzling feature of T cell responses involves the potency of alloreactions. It has been known for a long time that antigens encoded in the major histocompatibility complex (MHC) induce potent primary T cell responses, unlike virtually all other antigens. There have been a number of explanations proposed for this, among them the innate "potency" of MHC antigens as stimulators, the possibility that all IgV genes have specificity for species MHC antigens,[17] or the very high frequency of mature alloreactive T cells.[18,19] Whatever the explanation, it is clear from several different kinds of experiments that alloreactive T cells comprise a large proportion of all T cells, and those specific for a single haplotype may indeed comprise between 5 and 10% of the total T cell pool of an animal (Table 3). We must keep this startling fact in mind in evaluating other facets of T cell specificity.

Yet another interesting aspect of T cell responses to MHC antigens is the general (although not universal) finding that mutations in the K or D molecules of the MHC lead to very strong T cell responses between mutant and wild type, similar to those between independently derived haplotypes, but do not induce detectable antibody responses.[23] These mutations are sought using techniques that will optimize T cell responses, but there has been no intentional screening against antibody-detectable mutants. Although MHC mutants generally carry detectable serologic differences from

TABLE 3

Frequency of MLC-Reactive Cells in Rats

Combination	Estimated frequency	Ref.
BN PBL vs. BN×DA	1.3, 1.0, 3.5, 1.0, 0.5	20
AO TDL vs. AO	(1.0)	21
AO TDL vs. (AO×HO)	5.7	21
AO TDL vs. (AO×DA)	6.5	21
HO TDL vs. (HO×Aug)[a]	(1.0)	21
Lewis vs. DA	6.3	22
Lewis × DA (anti-DA idiotype)	0	22

[a] HO and August are identical at AgB, but differ at other H antigens.

TABLE 4

Anti-DNP Antibody does not Prevent T-Cell Response to DNP

Antigen	Proliferative response of T cells in presence of anti-DNP (as percent of response in normal Ig)
PPD	75 ± 5
DNP_{25}-BSA	123 ± 14
DNP-BSA-lipid	142 ± 12
DNP_8OVA	114 ± 16

Note: n = 4.

Data summarized from Janeway, C. A., Jr., *J. Immunol.*, 122, 1482, 1979. With permission.

wild type, the finding that such mutants do not elicit antibody responses in wild type but do induce very strong alloreactivity, is interesting in the context of the present discussion.

Finally, studies in which attempts are made to block the in vitro stimulation of T cells by antigen are of interest. First, antibody to antigen does not block T cell proliferative responses.[15] An example of this is the response to the hapten DNP in guinea pigs as shown in Table 4.[24] This is an important example, since in this system there is ample evidence for involvement of the hapten in the antigenic determinant, and the antibody is pure anti-DNP that could be shown to block expression of DNP on the presenting macrophage surface. Secondly, free antigen has not been successfully used to inhibit such responses. Thirdly, anti-idiotypic antibody will not block T cell proliferative reactions, at least in the system of Binz and Wigzell,[22] unless the cells are also treated with complement and thus killed. However, one type of antibody has consistently blocked T cell responses to antigen, and that is antibody to I-region determinants, a finding first reported by Shevach and Rosenthal.[25] This finding has been confirmed numerous times in other systems and can now be accepted as a general finding in T cell responses of the proliferative type.

In summary, it is clear from these examples that T cell responses to antigen show both similarities to and differences from B cell responses to the same antigens. We will return to these different examples of T cell responses later in this paper and try to explain them. Before doing so, four recent advances in understanding T cell responses will be covered:

1. Subpopulations of T cells, their functional and antigenic characterization
2. The role of MHC antigens in T cell responses
3. The role of Ig genes in T cell receptor formation
4. Antigen binding by T cells and T cell-derived molecules

III. SUBPOPULATIONS OF T LYMPHOCYTES

Shortly after the division of lymphocytes into T cells and B cells, subpopulations of T cells were discovered. These were initially defined by means of functional parameters, the first two being synergy in the graft vs. host (GVH) reaction[26] and suppression by T cells.[27] Subsequently, many different functions of T cells were discovered, and in general, they could be assigned to different subpopulations by means of experimental manipulations. However, this approach has severe limitations. Further advances awaited the introduction of different strategies.

These strategies were provided by the discoveries of differentiation antigens of T cells by Boyse and his co-workers.[28] These antigens are detected with cytotoxic alloantisera directed at determinants found on some, but not all, T cells. These antigens are not found on other cell types, nor are they present in detectable amounts on pre-T cells, hence the name "differentiation antigens". Through the work of Cantor and Boyse,[29] a number of subpopulations have now been defined serologically and their functional activities explored. The purpose of this section is to describe the antigens, the subpopulations of T cells so far defined using antisera directed at these antigens, and then to discuss the roles of various types of T cells in regulation of the activity of B cells in the humoral antibody response. This system is chosen since so much of our information comes from such studies. It also illustrates the great complexity of the T cell network and demonstrates the advantages of studying well-defined subsets of T cells in trying to make deductions about their specificity.

A. Differentiation Antigens of T Cells

Lyt antigens — Thus far, antigens of the Lyt series have provided most of the information about T cell subsets. There are two well-characterized antigens, Lyt-1, the gene for which is found on chromosome 19, and Lyt-2,3, the genes for which are on chromosome 6. The latter is really two antigens closely linked genetically and co-expressed on the same cells. Therefore, for the present purposes, they will be considered as one. Most thymocytes have both Lyt-1 and Lyt-23 on their surface, and can therefore be called Lyt-123 cells. Peripheral T cells fall into at least three groups based on the Lyt antigens: Lyt-1^+,23^- cells (Lyt-1), which make up about 35% of peripheral T cells; Lyt-1^-,23^+ cells (Lyt-23), which make up 5 to 10% of peripheral T cells; and Lyt-1^+,23^+ cells (Lyt-123), which make up about half of peripheral T cells. In addition, some peripheral T cells lack all three of the Lyt-1,2,3 antigens and may make up yet another subpopulation. The functions of these subpopulations will be discussed below.

Ia antigens — Ia antigens, encoded in the central portion of the MHC between K and D, are found almost exclusively on lymphocytes and macrophages. Initially, it was thought that B cells carried Ia antigens and T cells did not, but through the work of Murphy et al.[30] and others it has become clear that certain Ia antigens are found on subpopulations of T cells as well. In particular, Ia antigens coded for in the I-J subregion of the MHC are expressed on a very small percentage of peripheral T cells which, nevertheless, have very potent biological activities.

Qal — The antigen Qal[31] is found on about half of peripheral T cells. Work on cells bearing Qal has just started, and it is thus the least well defined of the differentiation antigens we will consider. The genes for Qal are tightly linked to T1a to the right of the MHC.

B. Subpopulations of T Cells Defined by Their Pattern of Expression of Differentiation Antigens

Two types of cells can be defined by means of their surface expression of differentiation antigens, (1) those with known biological functions and (2) those defined solely by antibody and complement-mediated lysis. For the present discussion, only the former are relevant. However, it can be anticipated that further subdivision of T cell sets will occur as more antisera become available.

Lyt-1 cells — Cells of the Lyt-1 class are involved in helping or inducing the functional activities of other cell types: other T cells, B cells, or macrophages. This function appears to be strictly linked to the Ly phenotype. The most striking demonstration of this is found in the experiments of Jandinski and Cantor[32] in which isolated Lyt-1 cells were cultured with the T cell mitogen Concanavalin A (ConA). These cells were always found to function as helper cells (Th) and did not express suppressor-T cell activity. Thus far, all purified Lyt-1 populations have given the same result. Nevertheless, there have been several reports of suppressive activity mediated by T cells that are Lyt-23$^-$.[33,34] This finding can best be explained in invoking feedback suppression, a phenomenon discussed in more detail below. The induction of feedback suppression is found within a unique subset of Lyt-1 cells bearing the antigen Qa1.[35] This subpopulation makes up about 60% of the total Lyt-1 population, but accounts for all of the feedback suppressor activity thus far observed. These Lyt-1,Qal$^+$ cells also contain helper T cells and act synergistically with the Lyt-1,Qal$^-$ subpopulation.[35] Other examples of synergistic interactions between helper T cells will be discussed later in this section. Thus, there are at present two families of Lyt-1 cells. Both express helper activity, and at least one is also capable of inducing suppressor T cells when mixed with populations of normal T cells.

Lyt-23 cells — Cells of the Lyt-23 phenotype are quite rare, making up about 7% of peripheral T cells. Nevertheless, they can be divided into at least two, and probably three, functional subpopulations. First, Lyt-23 cells are the cytotoxic effector cells (CTL) in most systems of cell-mediated lympholysis thus far studied.[36] Second, T-suppressor cells, that is, the effector T cells in suppressive phenomena, are also Lyt-23 cells.[29,37] However, these cells can be separated from one another on the basis of a cell-surface antigen coded for in the I-J subregion present only on the suppressor T cells and not found on CTL.[30] Suppressor cells (Ts) can probably be further subdivided into those that recognize antigen and those that recognize Ig-related determinants (see below).

Lyt-123 cells — These cells are very difficult to study since no reliable methods for preparing them as a pure population exist at present. Nonetheless, by comparing the activity of unfractionated spleen T cells with that of an artificial mixture of Lyt-1 and Lyt-23 cells, certain activities have been attributed to these fascinating cells. A note of caution should be inserted here to the effect that the conclusions reached in these studies are based on the assumption that the ratio of Lyt-1 to Lyt-23 cells is not critical when comparing a mixture of these cell types to whole-spleen T, and this assumption may not be valid. Nonetheless, the attribution of function to the Lyt-123 subpopulation is probably valid. Using this approach, Lyt-123 cells have been shown to do the following. They provide the precursors for T cells that kill either TNP-modified self cells[29] or that are induced to kill cells differing only in H-2K.[38] They are involved in the feedback loops of various kinds that will be described in the next section. About 50% of the Lyt-123 cells do carry the antigen Qal. The Qal$^+$ subpopulation contains those Lyt-123 cells involved in feedback suppression.[39-41]

C. The Regulation of an Antibody Response by Subsets of T Cells

The various subpopulations of T cells involved in regulation of antibody responses

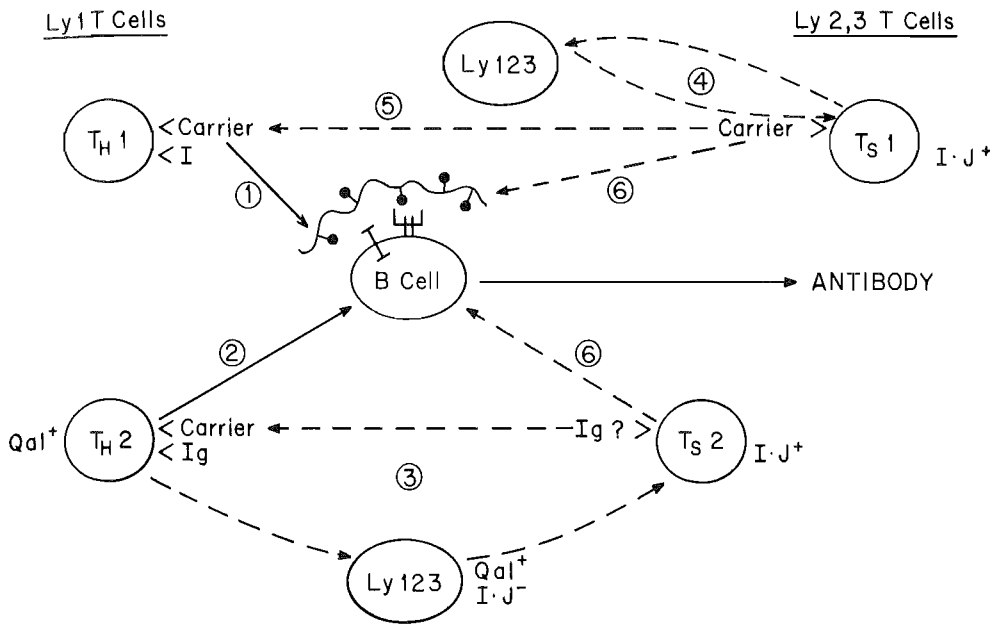

FIGURE 1. A minimal model of cells and their interactions during an antibody response. Solid arrows indicate helper interactions. Dashed lines indicate suppressor interactions. Several general features of this model should be noted at the outset. It omits macrophages, which play many important roles in T cell activation and in cell interactions. It shows but a single type of B cell, although subpopulations exist. It is pieced together from many, often nonoverlapping, sets of data. Thus, much is based on assumption and hypothesis. The numbered arrows demonstrate: (1) T_H1, a carrier-specific Lyt-1 helper T cell is essential for the induction of antibody responses. It requires hapten and carrier to be physically linked. (2) T_H2 recognizes B cells by their idiotypic determinants, and it will further induce B cells that have been induced by T_H1 cells. T_H2 requires an antigen-specific signal as well, but does not require hapten and carrier to be physically linked. (3) Feedback suppression loop. The cells in this loop must be idiotypically related. The loop is activated by T_H2 cells and requires both a Qal⁺, Lyt-123 cell and an I-J⁺, Lyt-23 cell. We have called this cell T_S2 and show it acting on T_H2. We believe this cell is analogous to the Ig-specific T_S described by Herzenberg et al.,[37] Eichmann and Rajewsky,[92] and Owen et al.[51] (4) T_S1 is an antigen-specific Lyt-23, T_S cell. It will bind to antigen on columns. Tada[123] has shown that such a cell can induce more T_S1 activity by interacting with an Lyt-123 cell, as shown by this loop. (5) T_S1 cells probably act by inactivating T_H1, since they are antigen-specific, as are T_H1. (6) It is possible that both T_S1 and T_S2 can also act directly on B cells. This point is not yet settled.

by B cells are shown schematically in Figure 1, the arrows showing interactions that do or may occur. To illustrate the functioning of these cells, the course of an antibody response to a hapten-carrier conjugate will be described, and relevant experiments cited to support the present model where appropriate. This model assumes that the B cell must bind the antigen in order for the antibody response to occur. In this instance, the B cell is binding hapten and the carrier is associating with other structures on the B cell surface. This does not imply that the Ig receptor on the B cell is necessarily involved in B cell activation.

Apparently, all hapten-carrier responses in vivo require the presence of a helper T cell (here called Th1) which recognizes an antigen that also binds to the B cell Ig receptor.[6] Exceptions to this rule may have been observed in vitro.[42-44] The difficulty in interpreting these in vitro results are twofold. First, are there contaminating Th1 cells whose activity is augmented by T cells which recognize and respond to antigen not linked to hapten; and secondly, are these effects relevant to the in vivo situation? From the point of view of specificity, these arguments are important only in determining which type of Th is being studied. This follows because many studies suggest that Th2,

which recognizes some part of the Ig receptor on the B cell and which can act synergistically with Th1 early in antibody responses,[7,45-47] can apparently be triggered by carrier alone, not linked to the relevant hapten.[116] If this is so, it would make sense of a diverse set of findings demonstrating the activity of two synergizing T-helper cells. Most of these reports suggest that both cells are antigen specific and must be activated by free carrier.[42,44,45] An exception would seem to be the recent report of Woodland and Cantor[46] in which cells primed with bovine gamma globulin (BGG) were able to help in the response to ABA-keyhole limpet hemocyanin (KLH) when KLH-primed helper T cells were also present. These authors concluded that the BGG-primed cell, which augmented the level of anti-ABA antibody bearing a cross-reacting or germ-line idiotype, was solely specific for the idiotype and had no antigen specificity. However, as the cells were prepared in fetal calf serum, which contains abundant BGG, it seems likely that this cell is similar to other Th2 cells in requiring antigen stimulation and received its stimulation from the BGG it was exposed to in vitro prior to transfer. We have recently been able to show a similar synergy between cells primed to ovalbumin and bovine serum albumin in the anti-DNP antibody response to DNP-ovalbumin, but only when BSA is also given to the recipients.[116]

It may seem unnecessarily complex to invoke two different types of Th, but several lines of evidence strongly support their existence (see above). It seems most likely that distinct subpopulations of Th cells have evolved to play different functional roles in the antibody response. Th1 is the essential cell, and Th2 greatly increases the effective activity of Th1. Th2 also appears to regulate the "quality" of the antibody response in that antibody class. Allotype, charge, and idiotype may all be regulated by such cells. A detailed consideration of the roles of these cells in the immune response will be published elsewhere.[117] Suffice it to say that Th2 exists as a unique subpopulation of Th cells, that they require Ig for their activation, and they would appear to recognize Ig on B cells during the induction of antibody responses. It is our opinion that this cell is also the inducer of feedback suppression from which it has not yet been discriminated, and that this cell acts early in immune responses, but is subsequently suppressed by the feedback loop labeled 3 in Figure 1.

Feedback suppression involves a signal given by a Lyt-1,Qal$^+$ cell to an Lyt-123,Qal$^+$ cell which, probably through an Lyt-23,I-J$^+$ T-suppressor cell, in turn suppresses the response, probably by shutting off the inducer cell (Th2). This, therefore, comprises a classical feedback loop. The effect of this loop is to decrease the net helper activity of the system by stopping the helper function of the Th2 cells. The recent finding that the cells in this loop must share VH genes to manifest feedback[118] strongly suggests that the communication between these cells involves idiotype-anti-idiotype interactions.[48] If this is so, it seems most likely that Th2 is primarily involved in recognizing certain idiotypes on B cells. These would be the so-called germ-line idiotypes, as suggested by the experiments of Bottomly et al.[47] and of Woodland and Cantor.[46] Since most of these idiotypes are found on antibody of relatively low affinity, the role of Th2 in affinity maturation would appear to be to induce the most common subset of B cells (those with low-affinity, germ-line-encoded idiotypes) early in the response. Following the activation of the feedback suppression loop by Th2 and the loss of Th2 helper activity that follows this activation, selection of B cells now depends entirely on Th1 which are specific for carrier (plus self-I-region products, see below). Only high-affinity B cells will carry significant numbers of antigen molecules on their surfaces, and thus, only such cells will be activated by Th1 cells. Whether such high-affinity B cells arise by selection of rarer VH genes, or via a somatic diversification, is also discussed elsewhere. Thus, if this model is correct, affinity maturation paradoxi-

cally appears to be a function not of the Ig-recognizing Th2 cells, but rather of the carrier-recognizing Th1 cells. It should be stated, however, that if high-affinity clones arise from low-affinity clones bearing germ-line idiotypes, then both Th cells will be required for affinity maturation to occur.

The eventual shut-down of the antibody response has been attributed to a variety of effects, among them the suppressive activity of serum antibody and the loss of stimulation following clearance of antigen by antibody. While both of these are important, we choose to stress here yet another mechanism that is called into play by the loss of free antigen. Ts1, the antigen-specific T-suppressor cell, can bind antigen directly and with sufficient avidity to be removed on antigen columns (see below). As the concentration of antigen in a system falls, Ts1 will more and more act upon cell-bound antigen and, thus, exert its suppressive effect either on B cells or on Th1 cells which are antigen specific and, thus, can be expected to bind antigen. Such cells have also been shown by Tada and his colleagues[49] to make a factor that can activate an Lyt-123 cell (Qal type not known) to make more suppressor T cells (Lyt-23). This is shown as loop 4 on Figure 1. Thus, as an antibody response continues and antigen is removed from the system the cells revert to an inactive state, but one that is set at a higher level.

In conclusion, T cells exist in a web or network of interactions of varying specificity which work upon one another to produce a net result (antibody). Attempts to speak of the specificity of a Th or Ts cell based upon experiments using mixes of unfractionated T cells may succeed but are difficult to interpret in the face of such a complex array of interactions between the cells. Thus, one must interpret most of the available data on T cell specificity obtained with unselected T cell populations with great caution. Far more precise answers will come from studies of isolated antigen-binding receptors, particularly when they are prepared from a single known subset of cells.

These findings leave us with a number of questions about the specificity of these different subsets of T cells, even in functional assays. For instance, what does Th2 see on Ig? Is this cell specific only for germ-line idiotypes or for all antigens on Ig, such as class and allotypic markers? Is Th2, as we believe, also specific for antigen, and if so, does it show the phenomenon of MHC restriction (see below)? Do Ts1 and Ts2 really differ in their specificity? The interesting results of Bottomly et al.[50] are still somewhat ambiguous on this point in that their Ts, presumably a Ts2 since it suppresses idiotypic antibody responses, is also carrier specific. On the other hand, the Ts2 of Herzenberg et al.,[37] apparently a very similar cell, does not manifest carrier specificity as far as has been determined (i.e., it does not need to come from an antigen-primed animal). Similarly, the idiotypic suppressors of Owen and Nisonoff,[51] which can bind idiotype, are said not to show carrier specificity. However, since they come from animals primed with antigen in complete Freund's adjuvant, and are restimulated with the same adjuvant, the mycobacterial antigen may serve as their specific antigen in this case. It may be that some Ts2 will show carrier specificity and others will not. On the other hand, carrier-specific Ts1 cells can clearly be defined by their ability to bind antigen. Finally, and in many ways most intriguing, is the question of the specificity of the various types of Lyt-123 cells. Are all such cells anti-idiotypic since they respond primarily to system-generated signals? This seems a distinct possibility for at least some Lyt-123 cells since the only antigen-specific signals the system can generate, that we know of, are idiotype or antigen-antibody complexes. All of these questions remain open, and answering them will be a formidable task unless a better technology is developed for isolating large numbers of specific T cell tumors or hybrid T cell lines which would make accessible large amounts of homogeneous receptor material.

IV. THE ROLE OF THE MAJOR HISTOCOMPATIBILITY COMPLEX IN T CELL SPECIFICITY

The major histocompatibility complex (MHC) of the mouse is encoded in genes found on the 17th chromosome and can be subdivided into a number of regions. Two of these are the transplantation antigens known as K and D, which are structurally and functionally similar molecules. Between K and D lies a region that controls immune responsiveness to a variety of antigens and also encodes for cell-surface molecules known as Ia antigens. These antigens are apparently potent stimulators in mixed lymphocyte culture,[52] whereas the antigens K and D serve as target antigens for cytotoxic T cells.[53]

The role of the MHC in the specificity of T cell responses can be addressed from a variety of points of view. First, the MHC serves as a potent antigen in T cell responses between cells of different MHC types. Indeed, it is called the MHC because incompatibility at the MHC is invariably associated with rapid graft rejection, strong mixed-lymphocyte responses (MLR), and potent induction of CTL. Secondly, the MHC plays a critical role in antigen formation, since essentially all T cell responses involve recognition of both the nominal antigen and a self-MHC antigen. This phenomenon has also been called MHC restriction. It suggests a biological role for the MHC which previous studies of transplantation biology could not provide. Finally, a number of observations suggest that the MHC may also be involved in the formation of receptor molecules, although this area is still controversial. We will consider each of these points in order, saving the last for a later section on antigen-binding molecules.

A. The MHC as Alloantigen

T cell responses between congenic mice that differ only at the MHC give rise to potent cytotoxic T cells and manifest marked proliferation on the part of the responding T cells.[53] MHC antigens also induce potent antibody responses directed at the glycoproteins on the cell surface that are involved in stimulation of these T cell responses. As mentioned previously, one of the puzzling features of T cell biology is the potency of these responses. Two types of explanations have been brought forward, only one of which makes logical sense and is supported by the available evidence. The first explanation is that these antigens are highly stimulatory or immunogenic. The difficulty with this definition is that we do not know what it means, since we do not yet know what determines immunogenicity in antigens. In this case, it cannot be "difference" from self, since some MHC mutant mice differ from wild type by a single peptide (point mutation?) and yet are potent immunogens. The other possibility is that there are many T cells available which are in some way primed to respond to these antigens. Indeed, one of the main differences between MHC antigens and non-MHC cell-surface antigens (known as minor histocompatibility antigens) is that primary T cell responses are seen with the former, while only intentionally immunized T cells will respond strongly to the latter.[54,55]

Thus, the strength of MHC antigens is due precisely to the existence of many T cells already primed or in some other way activated to respond to MHC antigens. Indeed, data cited in an earlier section of this paper strongly support this view. One should add that two types of experiment raise further questions about the nature of the T cell-MHC antigen interaction. First, it is known that MHC antigen presented to T cells on isolated cell membranes or on dead cells are at best only poorly stimulatory in primary in vitro culture.[56,57] Second, the recent experiments of Batchelor et al.[58] suggest that MHC antigens are only highly immunogenic in vivo when presented on living cells. Such cells need not be able to divide, but they must be viable. The implications

of these findings are not clear, but they do suggest that mere binding of MHC antigen is not an adequate signal to the T cell to divide or otherwise respond to that antigen. This, in turn, makes it even less likely that MHC antigens are uniquely immunogenic molecules as a result of their primary structures. Whatever the explanation of alloreactivity, no theory of T cell specificity that does not account for responses to MHC antigens can be considered successful.

B. The MHC Antigens in MHC Restriction

Two related phenomena have led to a general reawakening of interest in the MHC amongst immunologists. The first was the discovery that the amount of antibody produced in response to some antigens was controlled by genes mapping between the K and D loci of the MHC.[59] Delayed hypersensitivity responses were also shown to be controlled by these genes. The second was the finding that T cell responses, not only to these "genetically controlled" antigens, but also to virtually any antigen tested, required that the cell bearing the antigen also bear the MHC antigens of the responding T cell. This phenomenon, that T cells only responded to antigen "seen in association with self-MHC antigens," has been termed MHC restriction.[60]

MHC-restricted responses can be roughly divided into two types, those restricted by K/D antigens (i.e., those involving recognition of antigen in association with K/D products) and those restricted by I-region antigens. These two types of responses, in turn, appear to be mediated by two types of mature T cells, Lyt-23 cells and Lyt-1 cells, respectively.

Before proceeding with a description of recent experiments on MHC restriction, it might be worthwhile to review those discoveries that led to the general concept. Initial observations by Benacerraf et al.,[61] Miller and Mitchell,[62] and Mitchison[6] suggested that T cells could not interact with cells bearing antigen if the cells were not histocompatible. However, more refined experimental protocols were required to further exploit this system, and these first emerged in the studies of Kindred and Schreffler[63] who showed that allogeneic thymus grafts would not reconstitute T cell responsiveness in nude mice. Subsequent studies of Shevach and Rosenthal[64] and of Katz and his coworkers[65] demonstrated that T cell-macrophage or T cell-B interactions required sharing of MHC antigens. This could not be accounted for by allogeneic inhibitory effects.[66] Even more compelling evidence came from findings with F1 hybrids between genetic responder and nonresponder animals. F1 T cells had the responder phenotype, but would not recognize the genetically controlled antigen unless it was presented on cells (B cells or macrophages) with the responder parents' genotype. The interpretation of these experiments was relatively clear. It drove immunologists to consider the possibility that Ir genes can express their function in these non-T cells. This is now generally accepted, but the question of whether T cells also express Ir genes is still open. However, appreciation of the full meaning of this finding in immunobiology awaited further experiments on MHC restriction. Two sets of experiments were particularly revealing of this phenomenon.

The first set involved the development of a large number of systems in which the nominal antigen served as a target antigen for CTL in chromium-release assays, as well as in vivo tests of T cell function such as protection from viral infection. In these experiments, it was found that T cells from intact animals would recognize the nominal antigen only when it was on a target cell sharing MHC antigen with the CTL. These nominal antigens included viruses,[67] haptens,[68] minor histocompatibility antigens,[54,55] and tumor antigens.[69] The similarity of these results to those previously found using protein antigens was noted and various unifying hypotheses brought forward. Before proceeding to these hypotheses, it should be mentioned that the portion of the MHC

involved in target recognition in these systems was consistently found to be the K/D antigens, and not the I region.

Two explanations initially proposed to account for all of these findings were termed dual recognition and altered self. The dual recognition hypothesis stated that T cells recognized self-MHC antigens on the target cell by means of the T cell's own MHC antigens by a like-like interaction. Altered self hypotheses stated that the nominal antigen induced a change in self-MHC antigens, or vice versa, that was unique to both the MHC antigen and to the nominal antigen, giving rise to a new antigenic determinant, or NAD, which was recognized by a *single* receptor. It will be seen below that both hypotheses have aged poorly and that neither is adequate to deal with all of the evidence, though both contain at least part of the truth (and indeed, it must be admitted that the truth is not yet known).

Strict dual recognition died almost before it had been proposed, disproved by at least two different types of experiment. The first involved the responses of F1 T cells to nominal antigen presented on one or the other parental cell type. Dual recognition states that F1 T cells should recognize both, but the experiments showed that F1 T cells discriminated absolutely between the two antigen-carrying parental cell types.[67,68,70] Indeed, this was already implicit in the experiments of Katz et al.[65] and of Shevach and Rosenthal.[64] However, an even more telling blow to such theories came from studies of T cells raised from stem cells in the presence of foreign-MHC antigens. It would appear to be generally true that T cells differentiating in the presence of allogeneic MHC antigen-bearing cells acquire the ability to see foreign-MHC antigens as self, even though they do not bear such antigens on their surface membranes.[71-74] This is true both for K/D restricted CTL responses and for I-region restricted helper and DTH-proliferative responses. Results of this type clearly laid the strict dual recognition hypothesis to rest. What of the altered-self hypothesis? Up to the present time, no decisive experiments exist to rule this hypothesis out. However, the precision of T cell recognition of both self MHC and of the nominal antigen, and the great profusion of possible nominal antigens, render a strict altered self hypothesis (i.e., a physico-chemical alteration of self MHC) highly unlikely.

More revealing are the experiments on chimeric mice of Zinkernagel et al.[75,76] and Bevan.[77] F1 bone-marrow cells were transferred to irradiated recipients of either parental type and allowed to mature into peripheral T cells, whence they were challenged with antigen and tested for CTL specificity. A summary of these experiments is presented in Table 5 and Figure 2. These experiments demonstrated that the thymus in which a T cell matured determined which MHC antigens it could recognize as self. Since fully allogeneic chimeras are nonresponsive, a role of the T cell's own MHC may be inferred, although other, equally valid, explanations for this finding do exist. The current view of such experiments, which have been repeated by Sprent[78] for helper T cells, would state that thymocytes, upon contacting self antigens in the thymus, are induced to proliferate and differentiate into mature T cells. Only those cells whose receptors match the thymus at the MHC will be so activated. In all of these studies, it would appear that I-A is the locus that must be shared. T cells derived from chimeras in which the stem-cell donor does not share I-A with the thymus of the recipient do not respond to any antigen yet tested. This, in turn, has suggested the existence of T cell-macrophage or T cell-T cell interactions in the generation of helper or killer cells. In the killer-cell system, a helper T cell that was programmed to see I-A determinants on the precursor of the killer T cell would be able to recognize recipient, but not donor, I-A. Thus it could not help the donor-derived killer-T cell precursors. This leads to the result of no killer T cells being generated. This might be overcome if the antigen were itself a cell carrying host-MHC antigens.[74] Similarly, if helper T cells need to see

TABLE 5

Comparison of (A × B) F1 into A Chimeric T Cells with A T Cells

		CML-mediated by T cells from:	
Stimulator	Target	(A × B) into A	A
A	A	–	–
A virus	A	–	–
A virus	A virus	+ + +	+ + +
A virus	B	–	–
A virus	B virus	–	–
B	B	–	+ + +
B virus	B	–	+ + +
B virus	B virus	–	+ + +

Note: A and B represent strains that differ throughout the MHC.

After Zinkernagel et al.[75,76]

the antigen in association with macrophage I-A in order to become activated, and all of the macrophages are derived from the donor of the bone marrow in these chimeras, then it will not be possible to activate helper T cells in chimeras in which the donor and recipient differ at I-A.

The thymus of the recipient in chimera studies determines which MHC antigens will be seen by the donor cells as self, since F1 mice depleted of T cells by thymectomy and irradiation will be reconstituted by giving an irradiated parental thymus. T cells in such animals behave like those of the donor of the thymus graft.[75,76] That is, the radioresistant portion of the thymus in some way positively selects those precursor T cells that can recognize its MHC antigens as self. This positive selection exerted by the thymic epithelial cells can be differentiated from the negative selective process by which strong reactivity (analogous to alloreactivity) to self is eliminated or suppressed. This is so because, in chimeras of the type shown in Table 5 and Figure 2, the T cells recognize A as self, but are also tolerant to both A and B, even though B is not represented on the thymic epithelium. Thus, positive selection for self recognition by T cells occurs on the thymic epithelium, while negative selection can occur independently on the MHC antigens of thymic epithelium. This, in turn, suggests that the negative and positive selective processes occur independently of one another, and require different explanations. The fact that tolerance can be induced by injection of cells into neonatal animals likewise may have the same implication.[80]

These experiments are very revealing of the mechanisms by which T cells mature and are selected, in that they show both elimination of potentially harmful autoreactive T cells and the positive selection of functional, self plus antigen-reactive T cells. They do not tell us the mechanisms by which these effects occur. The findings of Weissman and his co-workers[119] of large amounts of I-A antigens on thymic epithelial cells is consistent with a cell-interaction model leading to induction of T cells with specificity for self I-A antigens. However, none of these experiments points clearly to an explanation for the finding of very large numbers of alloreactive T cells in all studies. There have been several explanations for this finding given in the past. The leading explanations would seem to be the following. Either the germ-line V genes used for the production of T cell receptors are rich in antispecies MHC reactivities (Jerne[17]), or allo-

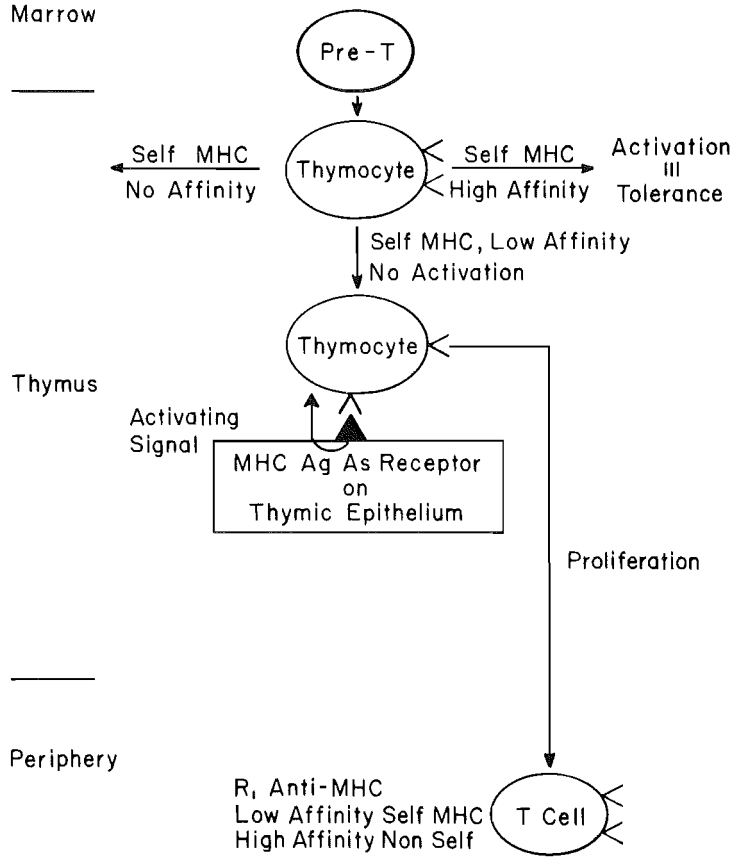

FIGURE 2. A possible interpretation of the chimera experiments of Zinkernagel and Bevan. Pre-T cells enter the thymus and express receptors. Those bearing strong antiself receptors are inactivated, the mechanism being that receptor-directed activation in the thymic microenvironment at this stage of differentiation leads to inactivation of the cell. Those cells with a receptor binding self-MHC-antigen on a thymic epithelial cell, but where binding is not sufficient to trigger the T cell (which would lead to activation/inactivation), instead act as ligand for the MHC-determined receptors in the TE cell. This TE cell then induces, via a factor or other short-range signal, the T cell bound to it. These induced cells will become mature T cells with the property of binding to self-MHC without becoming activated. Activation of such cells in the periphery requires binding of both self-MHC and nominal antigen by its two receptor sites.

reactive T cells are heteroclitic antiself-MHC-reactive cells.[19,60] The former model puts constraints on the V gene pool, either in its totality or in the V genes available for T cell receptor formation. The latter explanation requires no such constraints, although it is compatible with such constraints if they exist. The big difference between the two types of theories rests in whether or not somatic diversification of T cell receptors is an important part of T cell receptor formation. The arguments for or against this view will be given later in this chapter. A recent modification of Jerne's hypothesis,[81] to account for self recognition by T cells, suggests that cells with self-recognizing receptors are triggered to divide by thymic epithelium and continue to do so until one of the receptors diversifies. Therefore, the cell is no longer autoreactive, since it has only one valence specific for self.[81] This model does not predict separate processes for pos-

itive and negative selection. Until more evidence on the structure of the T cell receptors is available, such models remain speculative.

The specificity of self recognition would seem to be fairly complete. Thus, the chimera studies clearly imply that F1 stem cells can distinguish between one parent and the other once they have matured in the thymus. There is little or no cross-reactivity between different MHC antigens modified by the same nominal antigens, except in the TNP system. Even here, different sets of precursors exist in F1 T cell pools reactive to TNP-modified cells of one or the other parent.[70] Furthermore, most studies of MHC-restricted T cell responses show that, even when T cells are tolerant of a given allogeneic specificity, these T cells cannot respond to the nominal antigen associated with the tolerated alloantigen. Still, exceptions to this rule have been reported.[82,83] The resolution of conflicts of this type that exist in the literature will probably require further dissection of the responding cells into purified subsets of T cells, as many of the results reflect the effects of interacting helper and suppressor T cells.

The relationship between alloreactive T cells and T cells reactive to nominal antigen has received little attention. A very enlightening experiment, albeit requiring confirmation, was performed by Heber-Katz and Wilson.[84] These authors found that cells positively selected for alloreactivity could still serve as helper T cells for an in vitro antisheep RBC antibody response by syngeneic B cells. Since in mice, this response is clearly MHC restricted,[78] this result suggests that cells that are alloreactive can also be reactive to self plus sheep RBC antigens. One must, therefore, postulate either: (1) two different receptors for MHC antigens on one T cell,[85] (2) one receptor that reacts to alloantigen strongly, and to self MHC only when self is associated with sheep RBC antigens, or (3) the antiallogeneic MHC receptor cross-reacts with sheep RBCs. There are numerous difficulties with this intriguing experiment, amongst them the lack of proof that suppressive influences did not affect the specificity of the selection for alloreactivity, the lack of evidence for MHC restriction of the antisheep-cell response in the culture system used (indeed, there is evidence that it is not restricted[86]) and the possibility that sheep RBCs do cross-react with the selecting alloantigen. This system needs further exploration, particularly in mice where the characterization of the cells involved and MHC restriction of such cells is further advanced.

The recognition of MHC restriction as a general rule of T cell antigen recognition has led to a reinterpretation of a wide variety of experiments, most especially those involving Ir genes. These genes control responsiveness to a variety of antigens, above all those of limited complexity (e.g., antigens comprised of limited numbers of amino acids or evolutionarily related proteins such as the Ig allotypes). These Ir-gene-regulated responses are of T cell type, i.e., they involve helper-T cell function or delayed hypersensitivity responses. T cell from an F1 animal made by crossing a responder and a nonresponder strain are genetically of responder type. It came as a surprise, then, when Shevach and Rosenthal[64] and Katz et al.[65] showed that these T cells would respond to antigen presented by responder parental cells, but not to antigen on nonresponder parental cells, as mentioned previously. This was true for presentation by both B cells and macrophages. Thus, it appeared that Ir genes had their effect at the level of antigen presentation. The discovery of MHC restriction has led to a synthesis that states that Ir genes function as a special case of the general rule that T cells recognize antigen in association with self-MHC antigens, and that Ir genes are self-MHC antigens. The specificity of Ir gene effects is not yet fully understood. It may be that Ir genes represent a primitive antigen-recognition system and are involved in binding antigen. In this view responder animals would have Ir-gene products lacking the ability to bind the genetically controlled antigen.[60] Another possibility is that nonresponsiveness involves self tolerance.[87] In this model, T cells of animals do not discriminate

between their Ir-gene product associated with a nonpolymorphic self-cell-surface antigen to which they are therefore tolerant and the same Ir-gene product associated with the genetically controlled antigen. This model of Schwartz is accurately predictive and is appealing in that it does not require an antigen-recognizing capability on the part of the Ir-gene product. Its full correctness requires further testing. Finally, Ir genes may also function directly at the T cell level, either as self markers for T-T interactions or as part of the T cell receptor itself (see below).

One further issue that has, as yet, only begun to be examined is the question of how, and even whether, antigens associate on the surfaces of cells. Cohen and Eisen[88] have provided an interesting thermodynamic model, but direct verification that it is the physical association of antigen with self-MHC antigens that is recognized by T cells is lacking. In virus systems, Bubbers and Lilly[89] have provided provocative evidence that this association does occur. Viruses budding from cells carry with them the K and/or D products of the infected cells, and the product carried correlates with the product that T cells from the infected animal recognize. Similar findings of Zarling et al.[90] in cocapping experiments suggest that T cells see virus only in association with those MHC products with which it physically associates. Recently, Peterson and his colleagues have shown a physical association of a tumor antigen with a K/D-like molecule in a tumor cell.[91] The fact that a variety of viruses and minor H antigens are recognized preferentially or exclusively in association with certain MHC antigens is also most simply explained by selective physical associations of these molecules. However, the alternative explanations are numerous, and extensive biochemical data are the most likely to resolve the issue.

Teleologically, MHC restriction makes excellent sense. A T cell can most effectively combat a virus infection if it can kill virus-producing cells. Antibody is better suited to virus neutralization, since many molecules of readily diffusible neutralizing activity can be produced by one B cell. In order that the T cell home properly to its target, without being diverted by free virus, it must be nonresponsive to virus by itself. If T cells can only recognize virus plus self-MHC antigen, then they will only react with viral antigen on the surfaces of virus-bearing or infected cells, which is, indeed, apparently the case. Likewise, the Ia antigens serve as markers of cells of the lymphoid system and allow T cells to interact with the appropriate cell types during various immune responses, since antigen is only seen in association with a particular Ia antigen. Thus, helper T cells recognize antigen plus self I-A antigens which are found on macrophages and B cells, the two cell types important for this type of T cell, the former for its activation and the latter for its function. T cells that did not show MHC restriction would not be able to target their activity accurately within the system and would be functionally ineffective.

We hold to the view, previously expounded,[60] that alloreactive T cells are generated in such profusion because they represent heteroclitic reactions of cells that are, in fact, self-MHC plus nominal-antigen reactive. The positive selective force for alloreactivity, which must be invoked to explain the high frequency of alloreactive cells, is exactly the driving force that selects for self-reactive T cell clones.

V. ROLE OF IMMUNOGLOBULIN GENES IN T CELL RECEPTORS

As stated at the outset of this chapter, one of the problems that has plagued and perplexed immunologists since the discovery of T cells is the absence of readily detectable Ig on the surface of T cells. If T cells do not use Ig for antigen recognition, what do they use? Also, if they do not use Ig, why has nature devised two different, highly diverse and specific antigen recognition systems? Fortunately, these questions can now

be answered in fairly straightforward fashion by declaring the T cells and B cells use the same genes to generate their antigen-binding receptors. However, we do not yet know the overall structure of the T cell receptor. Therefore, we cannot yet state clearly how these genes are assembled in the T cell, which in turn might tell us why Ig is not *readily* detected on the T cell surface nor, for that matter, in its extracted membranes.

Ig genes play two important roles in T cell specificity. Most importantly, VH genes encode the antigen-binding portion of the T cell receptor. Also of interest is the recent finding that Ig is recognized by T cells, and that such T cells influence the quality of antibody produced in response to a variety of (and perhaps all) antigens. Furthermore, such Ig-recognizing T cells probably have further roles in immunoregulation that are as yet not described.

The evidence for involvement of Ig-VH genes in the formation of antigen-binding receptors on T cells comes largely from studies using anti-idiotypic antibodies raised against conventional immunoglobulin molecules or against T cells. Several sets of experiments have led to essentially similar conclusions, and the results have recently been summarized by Binz and Wigzell,[22] Eichmann and Rajewsky,[92] and Krawinkel et al.[16] These three groups of investigators have examined different types of receptors. Binz and Wigzell,[22] following on the earlier work of Ramseier and Lindenmann,[93] raised antibody against either antibody or T cells directed at foreign-MHC antigens, primarily in rats. Their studies have demonstrated the following about T cell receptors for these alloantigens. (1) They are expressed clonally on cells responding to the specific alloantigen and are not found on cells that do not respond to that alloantigen, nor on cells responding to other alloantigens. (2) They are found on approximately 6% of all T cells in a given animal. Their expression is masked in F1 hybrid rats between the responding and stimulating type. In F2 animals, of those progeny that lack the stimulating MHC type (which can therefore express T cell reactivity to that antigen), only rats carrying the allotypic markers of the heavy chain of the responding strain also carry the idiotype, suggesting a linkage of T cell idiotype to conventional Ig-CH genes. From this, the conclusion may be drawn that conventional Ig-VH genes are used to produce the T cell receptor. Furthermore, absorption experiments using alloantibodies have shown that isolated H chains, but not isolated L chains, will absorb the anti-idiotype. Finally, studies by Eichmann and co-workers[92] have shown that anti-VH antibody, but not anti-VL antibody, will react with helper T cell receptors. (3) In biochemical studies, Binz and Wigzell[22] have shown that idiotypic, antigen-binding molecules are shed from normal T cells, and that these molecules have a molecular weight of about 70,000 daltons. Dimers, and smaller degradation products, were also found bearing the idiotype. It is not known what the native state of the molecule is on the cell surface. Extensive absorption studies using antibody to all the known isotypes of heavy chain, polyspecific anti-Ig reagents, and anti-L-chain antibody all failed to bind the T cell receptor. Furthermore, a variety of anti-MHC antibodies failed to bind to these receptor molecules. Similar results have been obtained by Krawinkel et al.,[94] although the latter were able to bind their T cell receptor molecules with antibody directed at the rabbit A allotypic markers, which are located in the VH region. Thus, the picture that is developing from these studies is of a T cell receptor comprised of an antigen-binding portion encoded in conventional Ig-VH genes with a constant domain that is exclusively expressed in T cells, and which we may call for the present, IgT. One of the puzzling features of these T cell receptors is that most of the anti-idiotypic antisera used to detect them react with conventional idiotypic antibody only as intact immunoglobulin. That is, the idiotype appears to involve both VH and VL chains, or at least a VH stabilized by some L chain. On the other hand, the T cell receptor reacts with these antisera in the apparent absence of a light chain. The resolution to this

puzzle awaits more detailed biochemical and immunochemical characterization of the T cell receptor molecules themselves.

Earlier, under T cell subsets, it was mentioned that Ig plays a role in the development of T cell specificity that may be analogous to the role played by MHC antigens in dictating MHC restriction. This is, certain helper T cells appear to behave in such a way that they will only help B cells bearing idiotypes to which the T cells have been exposed during their ontogeny. This area is just now being explored, hence little detail exists. However, certain experiments are worth describing. First, T cells derived from animals congenic at the Ig-CH locus (and also the Ig-VH locus) were shown to fail to collaborate with B cells producing IgG2a of the opposite allotype, although they would collaborate with B cells bearing the opposite allotype of other antibody classes. The mechanism of this failure has not been determined, but it did not obviously result from suppression of helper T cell function.[37] Secondly, helper T cells raised in the absence of Ig or B cells were deficient in one of two detectable helper T cell activities.[45] This finding strongly suggested a role for conventional Ig, either on B cell surfaces or on macrophages, in the generation of a particular class of helper T cells. Thirdly, T cells generated in the presence of antibody to idiotypic determinants failed to help efficiently for that idiotype, although they could synergize with helper T cells that were raised in the absence of anti-idiotype antibody.[46] Finally, helper T cells raised in the absence of a given idiotype as a result of a genetic B cell defect in (CBA/N × BALB/c)F1 male mice would not help efficiently for that idiotype, although their net carrier-specific helper activity was apparently normal.[47] All of these studies are consistent with the idea that helper T cells for a particular idiotype are generated by exposure to antigen in the context of that idiotype and are not generated in the absence of that idiotype (or allotype?). These findings are consistent with the idiotypic network hypothesis of Jerne,[95] while at the same time sharpening its focus on certain aspects of the mechanism of this network. For instance, they point to the possibility that conventional Ig may imprint helper T cells so that they will later recognize the same idiotypes on B cells (and, perhaps, on T cells as well). However, the inductive signal probably comes from the conventional Ig-B cell pool, and not from T cells, since in some of the above systems T cells were not known to be directly affected by anti-Ig reagents or by the CBA/N genetic defect. Thus, both Ig gene products and MHC gene products serve as critical self markers in the immune system, and may do so by analogous mechanisms. Both imprint on the T cells specificities for self-cell surfaces that must influence the apparent or functional specificity of the T cells when they become mature helper or effector cells. Since these two gene complexes are also thought to contribute structural elements to T cell receptors, one can appreciate the formidable task that will be involved in understanding the functional specificity of T cells, raised in a given environment, long after their molecular structure is unraveled.

VI. ANTIGEN BINDING BY T CELLS

Antigen binding by T cells, and isolation of antigen-binding molecules from T cells, have been studied by a number of authors. For the purpose of this review, certain critical studies will be focused on, as they shed light on how T cells recognize antigen. Similar to failures to demonstrate Ig on the surface of T cells in the conventional sense, it has also proven difficult to demonstrate antigen binding by T cells.

Some time ago, Rubin and Wigzell[96] noted that helper T cells pass through antigen columns that could remove B cells, and that passage of T cells over such columns actually enhanced the helper activity of the population disproportionately to the yield of T cells. Rubin[97] subsequently demonstrated that the cell being retained on the anti-

gen column was a suppressor T cell. Thus, Rubin showed that T cells differing in function also differ in their antigen-binding properties. T helper cells do not bind measurably to antigen-coated beads under Rubin's conditions, while T suppressor cells bind very tightly. Subsequently, similar observations were published by both Okumura and Tada[98] and by Taniguchi and Miller.[99] These authors went one step further and actually isolated the antigen-binding suppressor T cells. They characterized these cells in terms of their Ly antigen phenotype and their expression of surface I-region encoded structures. The cells were shown to be typical T suppressor cells, with the surface Ly antigen phenotype $Ly1^-,23^+,I\text{-}J^+$. They were enriched for suppressor activity by as much as 100-fold.

Similar findings have been obtained in studying binding of cytotoxic effector cells. The effector cells themselves, which have the surface Ly antigen phenotype $Ly1^-,23^+$, do bind to monolayers of the appropriate MHC haplotype (i.e., that to which they were raised).[100,101] However, precursors of these effector cells bind less well to these monolayers, and cells giving proliferative responses to MHC antigens, most of which have the surface Ly antigen phenotype $Ly1^+,23^-$, bind weakly or not al all to cell monolayers. This finding suggests two things. First, that different subsets of T cells may differ either in the avidity or the surface expression of their receptors, and secondly, that cells in different differentiation or activation states (precursor vs. effector) may also differ in their ability to bind to antigen. If this is so, then it raises questions about whether one should discuss the "T cell receptor" at all, or whether it would not be more appropriate to discuss T cell receptors, defining each type of receptor by the subclass of T cells from which the material was derived.

Despite the general failure to demonstrate antigen binding by T cells from the Ly1 pool, certain exceptions to this now exist. Amongst the more interesting of these are the findings of Swierkosz et al.[102] who were able to fractionate helper T cells by their ability to bind to macrophages coated with the appropriate antigen, provided the macrophages were also of appropriate MHC type (see above). Similar findings in the guinea pig[103] and in the mouse[104] in proliferation assays have also been made. This has led to the suggestion that such cells do not recognize antigen per se, but only antigen in association with MHC antigens on the surface of a macrophage. However, a distinct alternative has recently been brought forward by the studies of Lonai and his colleagues[105] who have demonstrated a marked increase in antigen-binding T cells following incubation of the cells in supernatant derived from monolayers of macrophages. Thus, the requirements for macrophage presentation of antigen may be manyfold, including secure attachment of antigen to the macrophage surface, antigen processing,[15] presentation of antigen in association with the appropriate MHC antigens, and induction of receptor expression on the T cells by factors released by the macrophages. It is not clear which of these effects are critical, and which are not, but all should be kept in mind until further studies are completed. While discussing the role of macrophages in presenting antigen to T cells, it is worthwhile to mention the studies of Rosenthal et al.[106] who have shown two steps in the binding of T cells to macrophages. The first step is species-specific and takes place independently of either nominal antigen or MHC determinants. There may be a second step that is, in fact, MHC-related.[120] There follows another step that requires the presence of antigen, immune T lymphocytes, and macrophages of the appropriate MHC type, and which leads to not only stable macrophage-T cell interactions, but also T cell activation. It is interesting to note that neither trypsin treatment of antigen-bearing macrophages nor antibody to the antigen can interfere with this process, suggesting that the macrophage-T cell interaction takes place in a protected site and that the macrophage can communicate antigenic signals to the T cell even after its surface has been stripped of the antigen. These

intriguing studies suggest that, following initial T cell-macrophage interaction, the macrophage expresses on its surface, where the T cell is bound, those antigens it has recently encountered. The T cell in turn expresses on its surface its antigen-specific receptor. We can think of this as an immunological poker game. When receptor matches antigen, the T cell binds and starts to proliferate. If the two do not match, the T cell departs to talk to another macrophage. Thus, the T cell may carry its receptor in a relatively occult form, and the macrophage likewise harbors antigens, waiting for the T cell to pay its call.

Another example of differential antigen-binding behavior by different subsets of T cells is found in the paper of Nagy et al.[107] They devised an ingenious experiment in which T cells were allowed to respond to a mixture of stimulators that differed from the responder at either the K or the I region of the MHC. The responding cells in the form of T blasts were then collected by differential sedimentation at unit gravity and stained for the stimulator antigen presumably bound to their receptors. It was found that cells of the Lyt-1 type bound I-region stimulator antigen, while cells of the Lyt-23 type bound K-region stimulator antigen. Both cells in this case bound antigen effectively, but it should be borne in mind that both cells were isolated in a highly activated state, and this behavior may not be typical of all Lyt-1 or Lyt-23 cells, but may be dependent on the state of activation.

Finally, a sizable number of studies have been carried out on antigen-binding materials isolated from T cells by a variety of techniques. These studies fall roughly into two categories and give interestingly divergent results. The studies of Binz and Wigzell[22] who isolate receptors from alloreactive T cells by means of anti-idiotypic antibodies, and those of Krawinkel et al.[16,94] who use absorption of T cells on antigen-coated nylon nets, both take advantage of the property of binding to study their isolated T-cell molecules. Both of these investigators have found the following properties for their antigen-binding molecules. The molecules bind specifically to the antigen and carry idiotypic determinants, the expression of which is linked to Ig-CH allotypic markers. This suggests that conventional VH genes are used to produce these antigen-binding molecules. Interestingly, Krawinkel et al.[16] have recently shown that the idiotype of their T cell-derived antigen-binding molecules does not change with hyperimmunization, while the B cell idiotype changes dramatically in the same animal. Indeed, animals which lack the light chain genes required for B cell idiotype production nonetheless produce virtually all of their T cell receptors for that antigen in the idiotypic mode. As mentioned, neither investigator has found evidence for conventional isotypic Ig markers from heavy or light Ig chains on their T cell-derived molecules. Finally, no evidence for MHC-encoded antigens has been detected on these molecules by either group.

A second set of data, obtained using antigen-binding material detected not by its ability to bind antigen, but rather by its functional activity, gives a different picture of the nature of these molecules. In these studies, which originated in the work of Munro and Taussig[108] and which have been extended recently by several workers, molecules with the ability to either help or suppress antibody responses have been isolated from carrier-immune T cells. These molecules, in all studies so far reported, will bind to antigen columns and show antigen specificity, both when placed on the column and when eluted from it. These molecules are produced in amounts so small that, up to the present time, molecular characterization requires testing of the product by functional assay. The molecular weight is in the same general range as that found by Binz and Wigzell,[22] although, as these latter workers have shown, it is difficult to determine the molecular weight on such molecules since they are subject to rapid proteolytic degradation. Recent studies of Germain et al.[109] have shown that such molecules carry

idiotypic determinants found on antibodies to the same antigen in mice. Unfortunately, this idiotype is found on antibody molecules from all strains of mice tested. Thus, the mapping of the genes responsible for the expression of these idiotypes has not been possible. It has been possible to demonstrate, by successive absorptions to and elutions from columns of either antigen, anti-idiotype, or anti-I-J antibody, that such molecules bind to all three of these columns, and thus, must be comprised of material derived from Ig-V genes (antigen binding and idiotype) and MHC genes (I-J, suppressive potentiality). Similar data have been presented by Mozes.[110]

The conflict in these two sets of data is interesting, but does not seem insurmountable. The work carried out with these latter T cell-derived molecules looked only at their functional activity. This may reside in a small fraction of the total idiotypic or antigen-binding molecules present. One could make the analogy to a population of antibody, all with the same specificity, but differing in isotype. IgM antibody may account for only 3% of the antigen-binding activity, but because of its superior ability to fix complement it may account for the vast majority of complement-dependent lytic activity present in an antiserum. Thus, depletion with anti-IgM antibody would not affect the antigen-binding activity of the preparation, but would drastically reduce its ability to lyse cells in the presence of complement. That such an analogy may have some truth to it is supported by the finding of Tada et al.[111] that spleen cells from B10 congenic mice, which are genetically incapable of being suppressed by an I-J-bearing T cell-derived suppressor-inducing factor, are in fact stimulated to produce more antibody by exposure to this factor. This suggests (but does not prove) that the "factor" is, in fact, comprised of a variety of materials of differing biological activities, and that removal of any one of these factors might have a far greater effect on the biological activity of the mixture than on its ability to bind to antigen. Similar results have been obtained in our own laboratories.[121] Thus, the differences in detection systems may well account for the differences seen with the different T cell molecules.

Taken together, these studies suggest that the characterization of the T cell receptor in chemical terms may be an arduous task because of its heterogeneity. This heterogeneity may exist at many levels, as it does in the B cell system. First, at the level of antigen binding and idiotype expression, the studies of Krawinkel et al.[16] suggest that such receptors are less heterogeneous than B cell receptors since only the latter can express a great variety of idiotypes. However, since recent evidence suggests the presence of multiple copies of V genes in the germ line,[112,113] a large variety of potential receptor specificities must be available at the germ-line level. Secondly, there is isotypic heterogeneity. There is no evidence for the presence of conventional Ig isotypes on T cells, nor on their isolated receptors. However, it is clear from the studies just cited that isotypic (and concommitant functional) heterogeneity does exist in these molecules.Just how many isotypes there will be remains to be determined. Finally, there may be heterogeneity in expression of the receptors on cell surfaces, including association of receptor molecules with other molecules to generate complex structures, analogous to the combinatorial complexity of antibodies deriving from the combination of different heavy and light chains. Thus, as in the antibody/B cell-receptor case, numerous T cell receptors need to be isolated and studied before we can talk confidently about general, and about particular, T cell receptor molecules. Again, the example of antibody suggests that either T cell tumors with functional activity or hybrid cell lines will most readily supply such information by providing a usable amount of homogeneous receptor to study.

VII. DISCUSSION

The question this chapter set out to address was how T lymphocytes recognize anti-

gen. Before this process can be fully understood, particularly at the organismic level, it is clear that the following facts must be established:

1. The precise nature of the nominal antigenic determinant being recognized
2. The association of the nominal determinant with various self structures, amongst them self-MHC antigens and self immunoglobulins. These associations may vary independently of the nominal antigenic determinant itself, depending upon other features of the intact antigen or its fragments
3. The pool of VH genes available to the set of responding T cells
4. The imprinting of the T cell specificity repertoire by various self molecules, including MHC products and immunoglobulins, and perhaps a whole host of other nonpolymorphic and therefore less well-characterized self products as well
5. The status of the various T cell subpopulations in the system at the time the nominal antigen is introduced

It is clear from the above list that even if we knew the structure of a T cell receptor, we might not have a very clear understanding of the process of antigen recognition by even one T cell, let alone the T cell system in an intact animal. Nonetheless, information derived at many levels has told us more than just how complex a process antigen recognition really is. It has also provided at least partial answers to a number of the puzzling findings listed in the first section of this chapter.

To return to the puzzle with which the chapter was introduced, there remain several explanations for the carrier effect and for those exceptional cases in which T cells can recognize hapten on a variety of different carriers. One of these would be the generation of suppressor T cells in most responses to haptenic determinants, whereas protein antigens preferentially induce helper T cells. However, we have no idea why this might bo so, and another class of explanations seems both more satisfying and more likely to be correct. If one takes the position, now amply supported by experimental evidence, that recognition of protein antigens involves the recognition of complexes of the protein and self-MHC antigens, and that these complexes form in very precise ways depending upon both the structure of the protein and the structure of the self-MHC antigen, then hapten-protein conjugates can be expected to generate complexes of hapten and self-MHC antigen that are largely unique for each protein, since the protein determines the hapten-self-MHC-antigen association. Thus, T cells recognizing a given hapten following immunization with that hapten-protein conjugate will respond to that hapten essentially only when it is presented on that carrier, or one that is very closely related to it. If this is so, why then should certain hapten-carrier conjugates lead to apparently hapten-specific responses? In order to answer this within the framework outlined above, one would have to postulate that these particular conjugates lead to hapten-self MHC complexes that are more variable than those generated by conventional hapten conjugates. That this may be the case is suggested by the nature of those conjugates that do lead to hapten-reactive T cells, namely lipid-rich antigens and autologous immunoglobulins. Both of these types of molecules will bind strongly to the surfaces of macrophages and macrophage-like cells that are essential for antigen presentation to T cells during the primary response. Lipid-rich compounds bind by means of hydrophobic interactions with cell-surface lipid and the immunoglobulins by means of Fc receptors. The finding of Dickler and Sachs[114] that Fc receptors and Ia antigens associate in some way on B cell surfaces would seem to favor this type of argument. However, before this hypothesis can be confirmed, biochemical evidence of the mode of association between various nominal antigens and self-MHC products at the cell surface must be determined at the molecular level. A third type of explanation, stating

that T cells cannot make receptors that react with haptenic determinants, would seem unlikely in view of the existence of hapten-reactive T cells as well as a variety of hapten-binding T cell products.

A second puzzle, referred to numerous times, is the potency of T cell responses to alloantigens. As first suggested by Simonsen[18] and confirmed by numerous workers, most clearly by Binz and Wigzell[22] using anti-idiotypic antibodies to fractionate T cells, the frequency of alloreactive T lymphocytes goes a long way to explain the potency of alloreactions. However, if this is the explanation for the finding, what is the selective pressure for so many alloreactive T cells in these mice? Could it be due to environmental factors, is it encoded in the germ line genes that determine T cell receptors, or are both explanations correct (or incorrect)? First of all, it is clear that environment can influence alloreactivity in T cells, since T cells can be made tolerant to alloantigens encountered under certain conditions. Secondly, it is clear from the chimera experiments of Zinkernagel and others that T cells can "adaptively differentiate" to recognize as self the MHC antigens encountered during their differentiation in the thymus. Furthermore, the induction by the thymus of T cells that respond to nominal antigen in association with self-MHC antigens is, thus far, the only known positive selective event in the differentiation of the T cell. While it is not clear how this process occurs, it can be stated that such a process should not depend on somatic mutational events to generate T cell diversity, since a great deal of evidence, and particularly that of Binz and Wigzell[22] and of Krawinkel et al.,[94] points to the inheritance of T cell idiotypes and to their invariance in a given animal as well as between genetically identical animals. Thus, it seems more likely that the process of selection for reactivity to antigen plus self that occurs in the thymus is a purely selective one acting on a population of preexisting V genes. This, in turn, implies that such genes contain a high proportion of anti-MHC receptors. Alternatively, the selection may be a relatively inefficient process, most cells not being selected. The few that are of appropriate anti-self specificity will both expand *and* account for the alloreactive pool. This argument is laid out in more detail elsewhere.[60] In any case, these arguments, like those of Wilson,[19] would predict that alloreactive T cells would manifest other specificities as well and would not be readily distinguishable from the T cell pool at large.

One other area of T cell responses that would seem to deserve further consideration is the finding of heteroclicity of T cells, and its implications. Heteroclitic antibody to the hapten NP, in which heteroclicity was first described, is found primarily early in antibody responses and marks the product of a germ-line V gene(s). Later in B cell antibody responses, other V genes are used to make antibody that is no longer heteroclitic. This shift does not occur in T cells from the same animals.[16] This, in turn, implies that B cells have access to either a different pool of V genes from which to assemble receptors, or that the B cells have the ability to somatically diversify their V genes during response to a given antigen, while T cells do not. If this is generally true, it may explain the rather puzzling observation that anti-idiotypic antibodies which bind to only a portion of antibody of a given specificity will, nevertheless, bind to all T cells or T cell products of that specificity.[16,109,110] If this does turn out to be a general rule, it will greatly facilitate the analysis of T cell receptor structure and specificity and will explain why anti-idiotypic antibodies have been such a useful probe for T cell studies. It would also explain, in part, the divergences in T and B cell specificity observed in numerous studies, since in fact, the same V genes could give rise to different products with different antigen-binding activities in the two sublines of lymphocytes. Further diversity could derive from the use of L chains in B cells and the use of MHC gene products or of two different VH genes in forming receptors in T cells.

VIII. SUMMARY AND CONCLUSIONS

Studies of T cell responses to antigen have given rise to a great deal of descriptive information, much of which needs to be reexamined in the light of the following findings:

1. T cells use conventional VH gene products to form their receptors. Unlike B cells, the T cells do not appear to use VL gene products in their receptors, nor do they express the full range of variability found in VH gene products in B cells.
2. T cells respond not just to antigen per se, but rather to antigen seen in the context of self-MHC antigens. The ability of T cells to recognize a given MHC antigen as self is learned by a double selective process during the differentiation of the cell. Negative selection removes T cells strongly reactive to self determinants, while positive selection induces differentiation in just those T cells having a specificity for self-MHC antigens identical to that found in peripheral T cells. The nature of these processes is not fully understood.
3. A surprisingly large percentage of T cells also reacts strongly to foreign MHC antigens. While some evidence exists that suggests that T cells reactive to foreign-MHC antigens and T cells reactive to self-MHC antigen in association with conventional antigen are overlapping sets of cells, this point needs further experimental confirmation. It is, nonetheless, of fundamental importance.
4. T cells consist of numerous subpopulations differing in both specificity and function, many of which can be detected only in complex cellular interactions. Thus, to speak of T cells is no longer sufficient. Activities must be defined as belonging to a particular subset of T cells. There is also some reason to believe that antigen binding by T cells differs not only with the subpopulation of cells, but also with the activation state of the cells (e.g., activated vs. resting).

All of these issues need to be resolved before an accurate picture of self-nonself discrimination and antigen recognition at the T-cell level can emerge.

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