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Perspectives and Commentaries

Thymus and Immunity—II. The Last Three Decades

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'The outstanding feature of the development of immunology in the last 10 years has been the recognition of the function of the lymphocyte and of the importance of the thymus in the immune process' [1].

ALTHOUGH, in the last few years, hardly an issue of a journal on immunology has appeared without a reference to T cells, this was certainly not so in the early 1960s when immunologists considered the thymus as a vestigial structure filled with incompetent cells, and a graveyard for dying lymphocytes. Many, in fact, did not consider the thymus capable of taking part in the immune processes. One group, for example, considered their 'observations as evidence that the thymus gland does not participate in the control of the immune response' [2]. Much of the early research on the thymus was concerned with its development and structure and its possible endocrine function, as discussed by Grégoire in the preceding survey [3].

THE EFFECTS OF THYMECTOMY

The thymus was known to be involved in the development of lymphocytic leukaemia in mice and thymectomy at 6–8 weeks of age prevented leukaemogenesis in high leukaemic strain mice as well as in low-leukaemic strains given ionizing irradiation or chemical carcinogens (reviewed in [4]). In 1951, Gross [5] discovered that injection of filtrated material extracted from leukaemic tissues of mice with spontaneous leukaemia would induce the disease in low leukaemic strains, but only if given in the neonatal period. The thymus was also involved in this case. In order to determine whether virus could multiply *outside* thymus tissue, experiments were performed in which mice were thymectomized *before* the virus was inoculated, i.e. immediately after birth. 'Subsequent mortality was very high in mice that had been thymectomized at 1 day of age, more than 50 percent of these dying between 2 and 4 months (whether they had been inoculated with virus or not), suggesting that the thymus at birth may be essential to life' [6]. This was totally unlike the situation in adult thymectomized mice which had never shown untoward effects or curtailment of lifespan. It was clear that mice without a thymus from the time of birth were susceptible to infection because, when kept in 'clean' conditions, the incidence of wasting and death was less. Examination, even before the onset of wasting disease, revealed low levels of blood lymphocytes and 'atrophy' of the lymphoid system [7]. Foreign skin grafts, even those from strains not compatible at the major histocompatibility complex, or MHC, and even skin from rats, grew luxuriant tufts of hair [8]. Antibody responses to a variety of antigens were impaired [9]. These results led to the conclusion that 'during embryogenesis the thymus would produce the originators of immunologically competent cells many of which would have migrated to other sites at about the time of birth. This would suggest that lymphocytes leaving the thymus are *specially selected* cells' [7]. Yet in spite of this conclusion, published in 1961, immunologists were still reluctant to accept the concept of an immuno-

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logical role for the thymus. Medawar, for example, suspected 'that we shall come to regard the presence of lymphocytes in the thymus as an evolutionary accident of no very great significance' [10]. The importance of the thymus in establishing immune competence was soon confirmed by others [11, 12], and subsequently using the newly available athymic nu/nu mouse strain [13].

TWO UNIVERSES OF LYMPHOCYTES

A separate line of investigation led Glick and his coworkers [14] to conclude that the bursa of Fabricius, a lymphoid organ of birds somewhat analogous to the thymus, seemed essential in early life for normal antibody-forming capacity. Szenberg and Warner, in 1962 [15], were the first to show a division of labour among lymphocytes in chickens: early thymectomy impaired cellular immunity and bursectomy humoral immunity. This suggested that in birds, in contrast to other vertebrates which do not have a bursa, differentiation of lymphocytes with distinct functions occurred in two separate sites. Since, in the mouse, neonatal thymectomy not only impaired cellular immunity but also antibody production to some antigens, the mammalian thymus was believed to fulfil the function of *both* the thymus and bursa. Yet an explanation had to be found for the observation that neonatal thymectomy in mice was associated with a marked reduction of lymphocytes normally found in those areas of the lymphoid tissues associated with cellular immunity (e.g. paracortical areas of lymph nodes and periarteriolar lymphocyte sheaths in spleen) and not so much in those areas where antibody formation normally took place (e.g. follicles and germinal centres) [16]. The solution to this puzzle came from a series of experiments not designed to solve it. A systematic study of the role of various cell types in the reconstitution of immune functions in immunoincompetent mice showed marrow cells to have no effects in irradiated mice. Thymus cells, however, responded to antigen by producing large proliferating pyroninophilic cells but no antibody. When given together with bone marrow cells some antibody was produced, though much less than that given by an equivalent number of spleen cells, but this was considerably and specifically enhanced if the thymus cells had previously been exposed to the same antigen in a first irradiated host [17–19]. This introduced the novel concept of 'thymus cell education' carrying with it two implications: (a) some cell in the thymus was potentially able to carry memory of its antigenic experience, and (b) some interaction occurred between educated thymus cells and marrow cells. With genetically marked cells, it was shown that 'the precursors of the hemolysin-forming cells were derived not from thymus but from bone marrow' [20]. This was the first unequivocal proof that T cells did not become antibody-formers but were required to help potential antibody-formers, or B cells, produce antibody, presumably through some form of antigen-linked recognition [21]. The demonstration of T and B cell cooperation led to a reappraisal of numerous immunological phenomena including tolerance, autoimmunity, genetically determined unresponsive states, 'original antigenic sin', immunogenicity.

MULTIPLE LYMPHOCYTE SUBSETS AND THEIR PRODUCTS

T and B cells could not be easily distinguished by morphological criteria alone. A major step forward was made when various cell surface components became identifiable by the use of specific antisera (and later by monoclonal antibodies). The existence of the Thy-1 antigen on T cells [22] and the high density of surface immunoglobulin on B cells [23] was a convenient way of distinguishing and separating T from B cells. It was soon found that T cells themselves could be further subdivided according to function and cell-surface markers: cytotoxic T cells [24] and suppressor T cells [25] were generally Lyt-2^+ ([26], now called CD8) whereas helper cells were generally L3T4^+ ([27], now called CD4). A plethora of other T cell subsets, such as various types of helper and suppressor cells, have also been postulated and in some cases identified. Suppressor T cells probably play a crucial role in regulating a great variety of immune responses, both cellular and humoral, in antigenic competition, in the induction and maintenance of some forms of immune tolerance and in the control of allergic and autoimmune reactions. They may also serve to control potential B cell autoreactivities. Unravelling the complex interactions occurring between various T cells, B cells and macrophages is a goal of cellular immunologists today.

The exact way in which lymphocytes differentiate from primitive precursors in the thymus is still largely unknown. Four distinct populations can be distinguished, according to the markers CD4 and CD8, double positive cells accounting for the largest and containing short-lived cells doomed to die in the thymus for reasons that are not yet clear. Included in the single positive population are mature T cells about to emigrate and in the double negative population, probably their immediate precursors [28]. A complex pattern is emerging among the double negative cells and work is in progress to unravel the genealogy of the various immunocompetent T cells from their progenitors originally derived from bone marrow, i.e. those cells which will rearrange their T cell receptor genes to form part of the mature T cell pool.

Largely as a result of the work of Mackaness [29], evidence accumulated for the release by sensitized T lymphocytes of factors which activated macrophages to kill some intracellular bacteria. Similar mechanisms are likely to be involved in resistance to a variety of pathogens and hence in protection. Among the factors produced by activated T cells are the lymphokines, γ-interferon, the interleukins (e.g. IL 2 [30]), IL 3 [31], IL 4 and other B cell specific lymphokines [32] and GM-CSF [33]. These lymphokines have a variety of target cells and are produced for a short period of time after activation. There is evidence of coordinated regulation of their synthesis but much more work remains to be done to unravel the molecular events involved.

INFLUENCE OF THE MAJOR HISTOCOMPATIBILITY COMPLEX

The major histocompatibility complex (MHC, also called H-2 in mice) is a series of genes, on chromosome 17 of the mouse or 6 in man, which code for glycoproteins present on the membrane of many, but not all body cells, in the case of class I genes (H-2K and H-2D in mice), and limited to some cells of the lymphoid system, notably B cells, dendritic cells and macrophages, in the case of class II genes (I-A and I-E in mice). Using an assay termed the graft-versus-host reaction in chick embryos, Simonsen, in the late 1960s [34] estimated the frequency of alloreactive T cells, i.e. cells reacting to foreign antigens coded by the MHC, to be at least 100 times as high as the frequency of cells reacting to other antigens. To account for the high frequency of alloreactive T cells, Jerne, in 1971 [35], proposed that the repertoire of T cell reactivities was composed of a set of germline v genes (those coding for the *variable* regions of T cell antigen receptors) which coded for structures essentially complementary to the MHC (H-2 in mice) alleles of the species. After entering the thymus, potentially alloreactive T cells, which form a large proportion of the T cell pool, would not be influenced. By contrast, T cells with self-H-2 reactivities would proliferate in response to H-2 structures present in the thymus. This must not be allowed to continue, for it was argued that such cells could kill self-H-2-bearing cells. Random somatic mutations in the genes coding for receptors to self-H-2 would accumulate and thus decrease the strength of self-H-2 binding receptors (negative selection). Hence, only T cells without self-H-2 reactivities would mature. These would have their receptors directed to non-MHC antigens and each specific set would thus constitute a much smaller proportion of the total T cell pool than alloreactive T cells. Because of this particular way in which T cell reactivities are selected, virtually all T cells directed to non-MHC antigens would retain some 'memory' of their anti-self-H-2 past and would recognise antigens which contained some measure of 'H-2-ness'.

This negative selection theory accounted for the high frequency of alloreactive T cells and actually predicted 'MHC restriction', a phenomenon which may indeed be considered as the major discovery of the 70s. This was made by Zinkernagel and Doherty, in 1974 [36], when they documented a requirement for MHC matching between cytotoxic T cells and their targets. They mapped the genes imposing the restriction in cytotoxic T cells (CD8 T cells) in the class I category. In the case of CD4 T cells, the restriction was mapped to class II. In what manner the CD4 and CD8 molecules govern this division is unclear and has been the subject of intense investigations (e.g. [37]). One possibility is that they increase the avidity of binding of T cells to their targets, CD4 having an affinity for class I and CD8 for class II, but the molecular details of such affinities have yet to be worked out. Whatever the case may be, T cells would have either one receptor directed to both antigen and the MHC, or two distinct receptors, one for antigen, the other for MHC. MHC components may thus be considered to act as signposts for T cell antigen recognition and there is indeed recent evidence for the binding of peptides by MHC molecules [38].

Other experiments by Zinkernagel and his colleagues in 1978 [39], using thymectomized and thymus-grafted radiation chimeric mice, gave data implying that H-2 restriction had been imposed at the level of differentiation of pre-T cells within the thymus. T cells emigrating from the thymus could then perceive antigen only if presented by antigen-presenting cells in association with the appropriate MHC restriction element identical to that on thymus stromal cells. Such cells are thought to be the cortical epithelial cells; they are presumed to bind incoming T cells with appropriate specificities [40], T cells with inappropriate receptors undergoing apoptosis. In contrast to their role in imparting restriction, thymus epithelial cells appear to play no role in inducing tolerance to self-MHC, or to antigens in association with MHC. The evidence suggests that intrathymic macrophage or dendritic cells of haematopoietic origin are involved in tolerizing T cells to self-components [40], but the *modus operandi* is unknown. It is likely, however, that deletion of self-reactive T cells occurs, as shown by recent work in which an anti-self-H-2 specificity detectable by a monoclonal antibody was found on 3% of immature T cells in the thymus of a particular mouse strain, but not on any mature T cells [41].

That MHC genes did indeed govern T cell antigen recognition had been apparent since the dis-

covery of MHC-linked immune responsiveness (Ir) genes [42, 43]. The immune response to a large number of antigens was found to be controlled by genes mapping in the I region of the H-2 complex. These genes governed the activities of T cells (e.g. delayed type hypersensitivity, *in vitro* induced antigen proliferation) or T cell-assisted functions like antibody production. After the discovery of MHC restriction, it was possible to envisage identity of Ir gene products and restriction elements: class II gene products governed the immune response of CD4 T cells and class I products influenced that of CD8 T cells. The exact mechanism of this control is still controversial and opposing views have been formulated to explain nonresponsiveness. According to one, some form of association between antigenic determinants and MHC gene products is essential for T cell recognition [44]. T cells recognize the 'associative' antigen and are thus MHC-restricted. A 'nonpermissive' interaction between an antigenic determinant and a particular MHC component leads to nonresponsiveness. An alternative view seeks to explain nonresponsiveness in terms of a gap in the T cell repertoire [45].

Several sets of genes, including MHC genes, predispose to a variety of autoimmune diseases. Some of these genes are not normally expressed on endocrine tissues but have been found on target cells in human autoimmune thyroid disease and several other disorders, such as type I diabetes. Since the chief mediator of abberant MHC gene expression is γ interferon, it has been postulated that a local virus infection leads to interferon production, aberrant MHC gene expression and activation of cells able to react to autoantigens because they are now presented with self-class MHC [46].

THE T CELL'S ANTIGEN RECOGNITION RECEPTOR

The major discovery of the 80s has been the elucidation of the nature of the T cell antigen recognition receptor (TCR) and identification of the genes which code for it. Use of monoclonal antibodies and T cell clones were instrumental in isolating the TR [47, 48]. It is a disulphide bonded heterodimeric glycoprotein composed of two chains termed α and β, and associated with a trimeric molecule, termed CD3. Its coding sequences were cloned independently in mouse [49] and man [50], by differential hybridization techniques followed by identification of rearranging genes. Two other genetic loci coding for a γ [51] and a δ chain [52] were identified but the function of T cells bearing such a heterodimer has yet to be clarified. Evidence soon become available to show that the TcR α-β heterodimer was entirely responsible for both antigen specificity and MHC restriction thus vindicating the hypothesis of the one TCR model directed to both antigen and associated MHC component.

CLINICAL IMPLICATIONS

Work on the immunological function of the thymus and thymus-derived T cells in the last three decades has improved our understanding of natural resistance to infections, rejection of foreign transplants, MHC-linked diseases, and diseases of immunological aberrations including autoimmunity. It has given us new insights into ways of developing new vaccines and manipulating the immune response. The pathogenesis of the acquired immunodeficiency syndrome (AIDS) could never have been unraveled in the absence of the recently acquired knowledge on the physiology of the T lymphocyte system. Yet much work remains to be done, for in spite of the tremendous growth of immunological knowledge and the proliferation of immunologically oriented journals, we still lack the capacity to immunize against many infectious diseases, in particular parasitic infestations and AIDS.

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