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ROLE OF THE THYMUS AND RELATED ORGANS IN IMMUNITY*

BY

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At the beginning of 1961, I think it is fair to state, the function of the thymus was unknown. To-day I believe that we have the basis for an understanding of its function on which experimental and clinical study will gradually build up a substantial structure of theoretical and practical knowledge. The thymus, at least in the child, is a large organ, and it is rather satisfying to feel that we are taking part in the elucidation of the function of the last major organ of the body to remain a mystery.

The thesis I wish to support is that in mammals the thymus is an organ in which primary proliferation and differentiation takes place of the lymphocytic cell lines which will subsequently be responsible for a large portion of the immunological activity of the body. In the chicken this function appears to be shared by another important lymphoid organ, the bursa of Fabricius, and there is a good deal to suggest that in mammals we have yet to determine where the activities that correspond to those of the avian bursa should be located.

The Mammalian Thymus

In discussing the functions of the mammalian thymus I shall necessarily have to be very brief, and I shall touch only three major topics—the development of the thymus, the results of experimental thymectomy, and the association of thymic lesions with autoimmune disease.

Development of the Thymus

In mammals the thymus arises from epithelium of the third branchial pouch. According to Auerbach (1961), the lymphoid cells characteristic of the thymus arise directly from the epithelial cells of the thymus primordium, but the adjacent mesenchymal cells are necessary to "induce" the change in quality. It is possible and probable that the origin of the thymus in this rather special fashion may have significance for its eventual function. Differentiation of the thymus into cortex with actively multiplying closely packed cells, and the medulla with its much looser packing and more lightly staining cells, takes place at an early stage.

In the young animal, cell proliferation in the thymus, as judged either by mitotic counts or by uptake of label into deoxyribonucleic acid (D.N.A.) is five to ten times more active than in any other lymphoid organ. Most of the proliferation takes place in the cortex, and

according to Metcalf and Ishidate (1961) mitoses are significantly more frequent in lymphocytes adjacent to the P.A.S.-positive phagocytic cells. In the normal mammal there are no germinal centres or lymph follicles in the thymus and plasma cells are very rare or absent. Mast cells are also very infrequent in the normal mouse thymus. One other aspect of thymic cytology that needs to be mentioned is the intense susceptibility of the organ to damage by stress whether due to infection, x-irradiation, or the administration of corticosteroid drugs. There is acute destruction of lymphocytes which may result in almost complete depletion of the cortex within a day or two. Even in the unstimulated thymus there is a good deal of cellular destruction, and most of the P.A.S.-positive cells contain fragments of nuclear material.

Circumstantial evidence of various types makes it virtually certain that a large proportion of the lymphocytes produced in the thymus pass to the blood circulation (see Sainte-Marie and Le Blond, 1958) and become indistinguishable from any other of the lymphocytes of the body. There is good evidence that lymphocytes from other organs can repopulate a damaged thymus, but it is not clear to what extent lymphocytes from the circulation enter a normal thymus.

Results of Experimental Thymectomy

Removal of the thymus from a half-grown or fully grown mammal has no demonstrably harmful effect. There is some controversy regarding how effective thymectomy is in curing myasthenia gravis—the only condition for which the thymus is surgically removed in young people—but there is no mention that I have been able to find of any immunological or other disability following thymectomy for myasthenia. There are, however, some poorly understood haematological conditions associated with thymic tumours and sometimes recognized only after removal of the tumour.

In mice, thymectomy at a few weeks of age greatly diminishes the subsequent incidence of lymphatic leukaemia, and, according to Metcalf (1962a), appears to increase general health and longevity. In strong contrast are the results reported first by Miller (1961), and confirmed in several other laboratories, of the effect of thymectomy on mice on the first day of life. Such mice appear normal for about three months and then rather rapidly develop a syndrome resembling that of runt disease, which is usually fatal. The characteristic

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feature after death is great involution of the lymphoid tissues.

The most striking functional change in mice thymectomized at birth is their subsequent ability to retain skin homografts or even heterografts of rat skin. This ability varies from strain to strain—for example, F₁ hybrids (AKxT6) retained grafts of C3H, C57B1, BALB/c, DBA/2, and rat for more than 25 days in from 44 to 92% of tests. The results with BALB/c were much less striking, only two out of nine grafts surviving from tests with C3H and C57B1. Martinez *et al.* (1962) obtained good survival of homografts in their system only if these were of the same H2 histocompatibility group.

In addition to the diminished activity of homograft rejection, mice thymectomized at birth showed a decreased ability to produce antibody. This has also been reported for rats and rabbits thymectomized in the first few days of life.

The level of lymphocytes in the circulating blood is lowered in mice and rats to a value round 50% of the normal. Lymphoid follicles are deficient in the spleen and there is a general poverty of development of lymphoid tissues.

When we combine these findings with other studies on various aspects of lymphocyte function we can formulate a hypothesis which covers the facts in a form very close to what has been adopted by Miller *et al.* (1962). We assume that the cells necessary for various functions concerned with the maintenance of bodily integrity and recognized by their capacity to mediate homograft rejection are descendants of cells which are differentiated for this function in the thymus. They normally begin to pass from the thymus into the circulation around the time of birth; the extent to which this takes place before birth seems to vary from one strain of mouse to another. These lymphocytes are seeded to the spleen and probably lymph nodes where they multiply and develop full immunological competence.

I have said nothing about the thymus in relation to the production of classical antibody because I believe that present information is far too scanty for any valid generalization. Later on I will emphasize that in the chicken the production of antibody seems to be unrelated to thymic function. For the time being, therefore, I shall continue to regard both mammalian and avian thymuses as being primarily concerned with what I like to think of as a surveillance function. On this view there are populations of lymphocytes which contain cells tuned to react to any alien patterns which arise on cell surfaces anywhere in the body. They will therefore be concerned with primary homograft rejection and homograft immunity and in different situations with graft-versus-host reactions. Everything suggests that there are antibodies, often of incomplete type, of the same specificity as the competent cells, which can be produced on appropriate stimulation of the clones concerned with the surveillance function. It is a problem of major interest to determine what the relation of such antibody is to antibody against classical bacterial antigens.

If, as I believe, the thymus is the site where proliferation and differentiation of lymphocytes into clones with definable immunological functions occurs, we must also endow it with another function—the elimination or inhibition of self-reactive clones. This has been recognized as a necessary part of any elective theory of

immunity for several years. Only recently, however, has evidence emerged that allows us to place this function in the thymus with some confidence.

Immunological Tolerance

There is now a very extensive literature on the subject of immunological tolerance and its bearing on this question of the elimination of "self"-reactive cells, but we can perhaps compress the essentials into two categorical statements. We can say first that if cells of foreign type can become firmly implanted in the body in embryonic life they are treated precisely like cells genotypically proper to the individual. Equally we can say that many self-components, even one so vital and universal as D.N.A., can under certain conditions function as antigens and provoke damaging immunological reactions. If we expand the implications of those two irrefutable statements, I believe that we reach the position (1) that immunologically competent cells (and the antibodies they may produce) can develop or manifest specific reactivity against a wide range of potential antigenic determinants—including many characteristic of the body as well as those not found in the body, and (2) that in the normal animal any cells with reactivity against antigenic determinants recognized as self by virtue of their presence in the internal environment will be inactivated in this respect or actually destroyed.

There has recently been a general trend to doubt the earlier view that the whole process of tolerance and self-recognition was completed in embryonic life. It now appears likely that homeostatic control persists into independent life and everything points toward the vital concern of the thymus in the process. If a region of lymphoid tissue exists in the mammal after birth in which differentiation and proliferation of lymphocytes takes place and those unsuited for function because of autoreactivity are eliminated, we should *a priori* expect it to have the following characteristics.

1. It should be shielded from the entry of extrinsic antigens. Otherwise cell lines which would be potentially useful later would be eliminated by contact with the foreign antigenic determinants.
2. Free multiplication of lymphocytes should take place in a situation in which they can move freely—that is, no fixed structures like germinal centres or stable accumulations of inactive lymphocytes should develop. If lymphocytic cells are to be, as it were, "checked over" for the presence of abnormal reactivity against any self-components in the thymus, they must have free opportunity to move about freely in the thymic environment.
3. Evidence of death and phagocytosis of unsuitable cells should be visible. If cells with forbidden activity are destroyed some means must be available for disposing of the dead cell and returning its components to the metabolic pool.
4. No development of functionally active antibody-producing cells—that is, plasma cells—should be observed.

Thymic Lesions and Autoimmune Disease

In making these points I have, of course, been describing the structure and behaviour of the mammalian thymus. There are, however, circumstances in which the characteristic morphological features of

immunology activity are seen in the thymus. In at least two examples of autoimmune disease the thymic medulla may show the formation of germinal centres, lymph follicles, and plasma cells closely similar to what we expect to see in a lymph node draining an area in which a homograft is being rejected.

In a lecture given recently at the Royal Society of Medicine (Burnet, 1962), I describe in some detail the behaviour of the mouse strain NZB/BL which develops spontaneously an autoimmune type haemolytic anaemia. In addition to the positive D.C. test, which has been our main diagnostic criterion, these mice show a wide variety of other lesions of types to be expected from autoimmune processes. In a large proportion of these mice the thymus shows enlargement of the medulla with germinal centres, incomplete lymph follicles, and accumulation of plasma cells and mast cells (Burnet and Holmes, 1962). Our data are incomplete, but we never find such lesions until about the time the D.C. test becomes positive. In older mice, particularly those killed when moribund, the thymus is greatly atrophied, but in these there is a disorganized medulla with many mast cells which we presume represents the site of "burnt out" areas of past lymphocytic proliferation. Of mice 8 to 12 months of age in which the cortex is still well developed about 80% (22 out of 28) show evidence of immunological activity in the medulla.

The significance of this activity in the medulla of the thymus is not fully established and it may be that we are laying too much emphasis on the finding. Nevertheless, at the present time Dr. Holmes and I believe that the thymus represents the region in which the process of autoimmune disease is initiated and that in the NZB/BL mice we have an experimentally accessible system in which it should be possible to test that hypothesis.

Myasthenia Gravis in Man

There are two related situations that I should mention—myasthenia gravis in man and the development of lymphatic leukaemia in the "high leukaemia" mouse strain AKR. Myasthenia gravis in an autoimmune disease characterized by the presence of an incomplete antibody in the circulation which can be shown to react with skeletal and cardiac muscle (Nastuck *et al.*, 1960; Beutner and Witebsky, 1962). The pharmacological nature of the effect on the muscle is still unknown, but this in no way modifies the essential finding that an autoimmune process is concerned. As is well known, myasthenia gravis is commonly associated with thymic lesions. In about 20% of cases there is a visible tumour, usually non-malignant in character. In 75% of the remainder there are germinal centres and lymph follicles, often very numerous in the medulla. The appearances resemble very closely those seen in the NZB/BL mice. They have not been described in any other human disease, but it must be emphasized that myasthenia gravis is the only condition in which the thymus is surgically removed, that no practical method for thymic biopsy exists, and the necropsy carried out at the end of a more or less prolonged illness will usually present only a grossly atrophied thymus, hard to locate in the anterior mediastinal fat, and almost never taken for histological section by the pathologist. I have suggested more than once that a thymic biopsy from an early typical case of systemic lupus would show the same sort of appearance, but so far I know of no such examination having been made.

Lymphatic Leukaemia in Mice

The second condition concerns the induction of lymphatic leukaemia in mice. In a "high leukaemia" strain like AKR, leukaemia arises spontaneously from genetic causes which may be associated with vertical transmission of a virus; in other strains not genetically prone to the disease leukaemia may be induced by administration of the Gross virus or by x-irradiation. It is a very striking finding that whatever the apparent exciting cause the incidence of leukaemia can be greatly diminished by early thymectomy. Metcalf's (1962b) studies indicate that the first appearance of leukaemic cells (as judged by transplantation to susceptible hosts) takes place in the thymus of AKR mice approximately two months before death from leukaemia.

The findings in AKR mice strongly incline one to consider that the thymic changes in autoimmune disease are primary rather than a secondary result of a process initiated elsewhere. The two alternatives can be stated as follows.

1. If the thymus is concerned, as we have assumed, with eliminating lymphocytic cells capable of reacting with antigenic determinants on "self" components, we must postulate two different kinds of response to antigen, depending on whether the cell concerned is in the thymus or has established itself in the spleen or elsewhere.

If a cell competent to react with antigen A meets that antigen in the thymus it will either be destroyed or have its capacity to react with A "permanently" repressed. Outside the thymus appropriate contact with A will set in motion the proliferative and antibody-producing activities characteristic of a positive immunological response. The first hypothesis to be considered is that as a result of a genetically based instability a small proportion of lymphocytes undergo somatic mutation (or some equivalent change) which renders them and their descendants insusceptible to one of the normal homeostatic controls. This means that an occasional individual amongst the rapidly multiplying thymic lymphocytes swings over into positive immunological reactivity while it is still in the thymus. If such a cell should have capacity to react with a body component present in the thymic environment and any other necessary requirements are fulfilled, the characteristic features of immunological proliferation will arise in the thymus. Descendant cells will pass to the general lymphoid organs. Here we must probably assume that the same switch to homeostatic resistance will render them resistant to inhibition or paralysis by high antigen concentrations.

To bring the hypothesis in relation to what is known about AKR leukaemia we may say that in both conditions genetic ability results in the emergence of somatic mutants in the thymus. In AKR the mutant cells are highly resistant to the normal homeostatic controls and eventually produce fatal leukaemia; in NZB/BL mice a minor change in responsiveness to rather similar controls is all that need be postulated.

2. The alternative hypothesis to account for the appearances in the thymus might take the form that mutant clones of lymphoid cells arise anywhere in the body and that proliferation in the thymus has just the same significance as the very similar lesions seen in the thyroid in some cases of Hashimoto's disease.

The mutant clones simply lack the normal quality of being inhibited by large concentrations of the antigen with which they react, and there is no special significance to be attached to their proliferation in the thymic medulla.

The first hypothesis has at least the advantage of calling for a wide variety of experimental approaches as well as holding out some promise for practical medicine.

The Situation in Birds

I have spent most of my time in discussing the situation in mammals, mainly because of the clinical implications of autoimmune disease. In our laboratory activities in Melbourne, however, the chicken has always played a major part, and in regard to my present theme of what I like to call the "first level" of lymphocytic development the avian findings are even more illuminating.

I should first make a few remarks about the distribution of lymphoid tissues in the chicken. The spleen is basically similar to that in mammals but there are no lymph nodes. Along the intestinal canal there are extensive accumulations of lymphoid tissue in the gut wall which are probably equivalent to the Peyer patches and the lymphoid tissue of the appendix. The thymus takes the form of a string of separate lobes on each side of the neck. In the young bird the histology of these thymic lobes is precisely similar to that of the mammal, but, according to Thorbecke *et al.* (1957), as the bird ages lymph follicles appear and the thymus takes on the appearance and perhaps some of the functions of lymph nodes. In addition there is a lymphoid organ of special interest, the bursa of Fabricius (see Diagram).

First and second levels of lymphocyte production in mammals and birds. This diagram illustrates the general idea that in organs of the first level—thymus and bursa of Fabricius in birds, and thymus and possibly some other lymphoid tissue in mammals—lymphocytes proliferate under intrinsic (non-immunological) stimuli. In the organs of the second level lymphoid-cell proliferation, including plasma-cell production, is wholly immunological in origin.

This arises from an epithelial outgrowth of the cloaca and develops in a fashion rather similar to what I described earlier for the thymus in mammals. Briefly, at about the thirteenth day of incubation the lining epithelium of the bursa develops a series of active points where epithelial proliferation leads to a nodule. As each nodule enlarges the cells in the centre change their character and become more loosely packed and primitive in appearance. From them a follicle of Stannius develops, a lymphoid unit very much resembling thymus

in structure. These eventually become closely packed to fill the plicae of the bursa, which are, however, still covered by a continuous epithelial layer.

The bursa is quite a large organ, approximately as large as the spleen from hatching to about 5 to 6 months of age. Thereafter it atrophies and is represented only by a fibrous remnant in the sexually mature bird. It had been suggested earlier that the bursa had an immunological function, but the first experimental approach came from work by Mueller *et al.* (1960). They showed that surgical removal of the bursa at 7 days of age gave birds which showed a greatly reduced primary antibody response when tested at 22 weeks. It was also shown, following earlier work by Meyer *et al.* (1959), that administration of 19-nortestosterone at the fifth day of incubation prevented the development of the bursa and that the rather sickly chicks so obtained had no capacity whatever to produce antibody.

Our own studies in this field (Warner and Burnet, 1961; Szenberg and Warner, 1962) have been concerned mainly with the use of testosterone as an inhibitor of lymphoid tissue developed in the chick embryo. The main effect is on the bursa, and by administration of 2 mg. per embryo at the twelfth day of incubation lymphoid development here can be completely prevented. The bursa then persists as a wholly epithelial organ. In 80% of treated chickens the thymus and spleen appear to develop normally. In this group Szenberg and Warner (1962) find that there is complete failure to develop antibody against a range of standard antigens and inability to develop delayed hypersensitivity to tuberculin. On the other hand, skin homografts are rejected in normal time and there is no change of the effectiveness of circulating leucocytes in producing graft-versus-host foci on the chorio-allantois.

A proportion of chickens show, in addition to an atrophic non-lymphoid bursa, complete atrophy of the thymic cortex. These birds are more sickly and rarely survive more than a few weeks. Limited experiments have been consistent in showing that such chickens retain skin homografts for prolonged periods in each case until the death of the bird from 12 to 24 days after grafting. There was, however, no significant change in the competence of the large lymphocytes of the blood to produce foci on the chorio-allantoic membrane. The indication, therefore, is that the thymus, as in the mammal, is in some way concerned with the function which can be demonstrated as homograft rejection. Szenberg and Warner (1962) have had three chickens with small amounts of lymphoid tissue in the bursa and complete atrophy of thymic cortex. These showed retention of homograft with a low level of antibody production. The results suggest that, if it were possible to remove all thymic tissue surgically on the first day after hatching, the chickens would show unimpaired antibody response with loss of the capacity to reject homografts.

When we look at these results we can hardly avoid drawing some rather far-reaching conclusions. Unless there is some unrecognized flaw in the experiments or in our basic assumptions we must conclude that there are certainly two and possibly three distinct systems of primary lymphocytes in the chicken, each responsible for providing descendant populations with a restricted area of immunological function.

(a) The bursa of Fabricius provides cells which will give rise to antibody-producing clones and in addition

to cells concerned with delayed hypersensitivity reactions against bacterial antigens.

(b) The thymus gives rise to cells whose descendants are primarily concerned with, one may guess, the maintenance of the chemical integrity of the body and which can be shown to be responsible for homograft rejection. They are also responsible for the development of lymph follicles or nodules in the spleen.

(c) The origin of the cells which mediate the Simonsen phenomenon is still unknown. In view of the known relationship of graft-versus-host reactions to homograft immunity one would expect such cells to be derived from the thymus. At the present time the most reasonable speculation is that the clones of cells concerned are derived from the thymus but very early find their optimal site for colonization and proliferation in the bone-marrow. This, it should be stressed, is purely a speculation based on two facts only—that the capacity persists despite complete inhibition of the thymic cortex and that circulating large lymphocytes are more effective than those from any of the lymphoid organs.

It will be noticed that the functions ascribed to the avian thymus correspond closely to those demonstrated for the thymus in mammals, and that the influence of the mammalian thymus on eventual antibody production is variable and may be largely indirect. This raises the rather urgent question of whether there is a functional equivalent of the bursa of Fabricius in the mammal.

Conclusion

I do not think it is too much to say that the past two years have seen a revolutionary change in the approach to immunology. Perhaps one can put it best by saying that we are concerned with complex biological functions and we must recognize that the rather naive ideas that have been advanced to explain immunity and antibody production must be replaced by a more sophisticated approach, the essence of which is that the development of the immunological competence of the body is part of the process of morphological and functional differentiation in the embryo and young animal.

This is not the place or the time to attempt any elaboration of this approach, but I should like to end with some very general remarks about the main points which will need to be covered by any future general theory of immunity.

In the first place immunology is concerned with the mechanism of protein synthesis, how genetically stored information is translated into specific protein structure—in our special case, of antibody. It is also concerned with the cells that are responsible for these syntheses and their day-to-day behaviour at the level of population dynamics in relation to the body as a whole. Such topics as the life history of the lymphocyte, the stimuli and environmental conditions which induce plasma-cell formation, and the nature of the memory cell of antibody theory belong here.

The next phase is the one I have been stressing in this lecture, the process of differentiation by which the potentialities of the fertilized ovum are segregated into progressively more and more specialized cell lines. For several years I have supported an elective view of the origin of antibody specificity (Burnet, 1959) as opposed to the old-fashioned view that antigen "instructs" each

cell to produce a complementary pattern, the antibody. If this genetically based elective view is to prevail, means must be developed to study the fine structure of functional differentiation between cell lines which must exist if there is to be material on which selection can work. The evidence from the chicken of preformed functional differentiation corresponding to thymic or bursal origin is perhaps a first step in this direction.

Another aspect of development and differentiation that has hardly been considered at the immunological level is the availability at every level of morphological development of some capacity to dedifferentiate and then to redifferentiate. Most of the published objections to a clonal selection theory of immunity are valid only if they are applied to a totally inflexible system without any such capacity for redifferentiation.

The final point I would like to mention in this brief survey of the needs of the new immunology is the relevance of the evolutionary angle. Immune phenomena, whether concerned with the recognition of foreign tissue cells or the production of antibody against microbial antigens, are limited to vertebrates. There is much scope for comparative study in invertebrates and the more primitive vertebrates of the primordial forms of both aspects. Here perhaps more than anywhere else we may be able to find the answer to the main problem that is presented by the work I have discussed in this lecture: whether in immunology we are dealing with two or more basically distinct sets of biological functions or whether it is still legitimate to seek unifying generalizations that will cover the whole field from antibody against viruses to autoimmune disease.

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A temporary adventure playground at New Place school, Shedfield, Hants, was a feature of this year's children's holiday organized by the British Epilepsy Association. Its object was to give the children—28 boys and 31 girls—the chance to play and build freely with the minimum of supervision. Most of the children come from large towns or densely built-up areas; about half attended special schools owing to their epilepsy (often combined with another handicap). Materials were supplied by a number of builders and timber merchants and hardware stores in Portsmouth and Southampton. The helpers were a group of theological students from King's College, London.