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THE ROLE OF THE THYMUS IN AUTOIMMUNE DISEASE

By

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During the last three years the thymus has been promoted to a position of singular distinction in the immunological hierarchy. At the same time it has been delegated the role of prime suspect as far as autoimmune disorders are concerned. This suspicion has arisen because of the clinical association between pathological changes in the thymus and diseases with autoimmune symptoms such as myasthenia gravis and systemic lupus erythematosus. These pathological changes present either as a thymic tumour and/or hyperplastic accumulations of lymphocytes or an occasional germinal centre in the thymic medulla. Weight has been added to the suspicion by the fact that mice of a strain known as the New Zealand Black (NZB) develop autoimmune haemolytic anaemia quite spontaneously (1, 11) and also show germinal centres and lymphocytic proliferations in their thymus (3, 4). Further, the hybrid progeny of NZB $\times$ NZW mice, according to *Helyer & Howie* (10, 11), develop lupus nephritis and have positive LE tests.

Now, while it may be reasonable to assume that there is an association between these events it is by no means clear whether the thymic lesions are a primary cause or a secondary effect of the autoimmune reaction. The problem can be considered in two ways—firstly how significant are the thymic changes and, secondly, what are the effects of removing the thymus from NZB mice and from mice of other strains.

Lymphoid follicles and germinal centres, when seen in the

spleen and lymph nodes, characteristically reflect immunological activity but they are not usually present in the thymus of normal animals. Thus, these germinal centres and lymphoid follicles seen in the thymuses of NZB mice could indicate the presence of abnormal clones of cells reacting to self-antigen. The available data for NZB mice as reported by *Holmes & Burnet* (13) show that 50 per cent of the animals give positive direct Coombs tests by the time they are 4–5 months old but that they die, on average, at 15 months so that the anaemia is not acute. Moreover, the time relationship between the appearance of Coombs positive tests and the development of thymic germinal centres is rather surprising. Male mice tend to become Coombs positive a few weeks earlier than females, but pathological changes in the thymus develop first in females. Further, *Holmes & Burnet* (13) record about 25 per cent of males with positive Coombs tests but no thymic lesions whereas the same proportion of females had thymic lesions but a negative Coombs test. The results in the female mice could be explained by saying that the thymic follicles in those animals which remain Coombs negative may be causing some other autoimmune manifestation but the development of Coombs positive tests in some males which do not have thymic follicles is troubling if we are to consider the thymus as the sole originator of abnormal clones of cells as suggested by *Burnet* (2, 4).

Our own work on the NZB mice has brought home to us very sharply that their thymuses are quite different histologically from those of the other strains with which we are familiar. For instance, necrosis of cortical cells occurs in mice only one month old and the normally obvious delineation between cortex and medulla is blurred but the first evidence of a definite germinal centre was seen in a female aged 12 weeks. The results of our Coombs tests agree approximately with the existing data. However, it is the effect of neonatal thymectomy on the NZB mice which has been our particular interest. *Helyer & Howie* (12) showed that removing the thymus on the day of birth does not prevent nor delay the

development of the haemolytic anaemia in NZB mice nor alter the incidence of LE tests in the NZB $\times$ NZW hybrids. In our experiments on NZB mice, thymectomized for various practical reasons 36 hours after birth, we find positive Coombs tests in some animals only 5½–9 weeks old. Thus, removing the thymus certainly did not prevent the conversion to the Coombs positive state and may even have precipitated it. These are only preliminary results and we wish to study possible artefacts very closely, but they are intriguing findings.

Another possible autoimmune marker, the Antinuclear Factor (ANF), has also been detected by *Norins & Holmes* (17) in unoperated NZB mice but there was no difference between males and females and the incidence could not be related to the presence or absence of thymic abnormalities. Caution is needed because the same authors discovered that ANF was present in 85 per cent of another very healthy outbred strain of mice, which never showed thymic lesions or positive Coombs tests. *Friou & Teague* (9) also checked 17 strains of mice and reported that the sera of "A" strain mice reacted with the nucleoproteins of both human and mouse nuclei but again these mice showed no obvious diseases or disabilities. So it would appear that pathological changes in the thymus are not an essential pre-requisite for the appearance of autoimmune symptoms.

As far as the effect of neonatal thymectomy in strains of mice other than the NZB is concerned it is now clear that some of the immune responses of neonatally thymectomized mice are impaired, that they show lymphocytic depletion, develop a wasting syndrome and die prematurely usually before they are 3 months old (16: 18–20). The aetiology of this wasting syndrome is still a contentious and difficult problem and it has been suggested that removal of the thymus at birth in some way incites an animal to destroy its own tissues and, in so doing, gives rise to the wasting syndrome (6). Because lymphocytic depletion is a characteristic of neonatally thymectomized mice it is pertinent to ask how such animals could execute an auto-immune reaction. Perhaps it is not so

generally recognized that the immune responses of such mice are selectively, not totally, impaired. Although they are unable to reject skin grafts, they are able to produce normal amounts of humoral antibody in response to primary stimulation with certain antigens even when they are already wasting (15). This is not altogether surprising since we regularly see plasma cells in their spleens and lymph nodes. Thus, a neonatally thymectomized mouse has the equipment to mount an autoimmune attack but in a total of 63 neonatally thymectomized mice of 3 strains we have not found evidence of ANF, LE latex nucleoprotein slide tests were negative and direct Coombs tests were also negative, with the possible exception of 3 animals. We would emphasize that all the 63 mice were wasting. On these grounds, therefore, we would hesitate to say that the wasting syndrome which follows neonatal thymectomy is a direct result of an autoimmune reaction although it is not beyond the grounds of possibility that autoimmune symptoms might appear coincidentally in such animals.

It is also possible to regard autoimmunity as a consequence of a breakdown in the tolerance which an animal normally shows towards its own tissues and so the induction and breakdown of tolerance are relevant to this discussion. Removal of the thymus does, in fact, extend very considerably the period of time during which tolerance to cellular antigens can be induced in mice. For instance, neonatal thymectomy makes it possible to induce tolerance in C3H/Bi mice by allogeneic (AKR) thymus grafts implanted as late as 14–17 days after birth. Mice so treated do not waste but they do accept and succumb to AKR leukaemic cells injected 6–8 months later. Suspensions of normal AKR spleen cells instead of thymus grafts can also be used to induce tolerance but one must bear in mind that, in these experiments, induction of tolerance towards the specific antigen, the AKR cells, is also accompanied by the general restoration of immunological reactivity (7).

Another example of prolongation of tolerance induced by neonatal thymectomy is shown by experiments using the virus

of lymphocytic choriomeningitis. This virus produces a fatal infection in adults but fails to kill newborn mice which survive with a persistent viraemia. It has been suggested that the newborn animals develop a form of tolerance to the virus and that the pathogenic effect of the virus in adults results from hyperimmune reactions of the host to the virus rather than from the cytopathic action of the virus on the host (14). We have found that neonatally thymectomized mice, unlike intact controls, can survive infection with LCM virus well beyond the neonatal period. In fact, they can survive infection given as late as 62 days of age although they become persistently viraemic (8).

Several groups of workers have been studying the effect of thymectomy in adult mice and the prolongation of tolerance towards the protein antigens, BGG and BSA (5, 21). The gradual breakdown of tolerance is significantly delayed if the thymus is removed from adults. Nevertheless, it is significant that some breakdown of tolerance did occur after thymectomy and it seems likely that some maturation of cells can continue even in the absence of the thymus.

It may seem that we have a contradictory situation in that thymectomy of neonatal or adult mice extends the period of tolerance, whereas neonatal thymectomy in the NZB mice apparently precipitates the breakdown of autotolerance. Can these contradictions be reconciled on a purely mechanistic basis? The histological picture of the lymphoid organs in neonatally thymectomized mice shows depleted areas which would normally be occupied by lymphocytes. This depletion would make space available in which other existing cell lines, or injected lymphoid cells, could proliferate. The amount of space depends upon the age at thymectomy—the earlier the thymectomy the greater the space available. Under these conditions it should be easier to induce and maintain tolerance with injected cells and, by the same token, any aberrant clones of cells which might arise in the NZB mice, outside the thymic orbit, would have an advantage.

Our comments have been deliberately restricted to those

situations in which thymic abnormalities are known to be associated with autoimmune symptoms. This tends to focus attention on a particular aspect of autoimmunity, namely, the action of immunologically competent cells, or of antibodies, against normal constituents of the body. It should not be forgotten, however, that sequestered antigens or abnormal antigen-antibody complexes can precipitate autoimmune reactions, or that viral "triggers" may also be implicated. Certainly, the role of the thymus in autoimmune disease should fall into better perspective as we learn more of its role in normal immunological development, but at the moment, and judging by experimental facts only, the thymus may be suspect but the case is by no means proven.

SUMMARY

Pathological changes in the thymus are associated with such autoimmune disorders as myasthenia gravis or SLE in man and Coombs positive haemolytic anaemia in NZB mice. The problem of whether the thymic lesions are a primary cause or a secondary effect of the autoimmune reaction was considered in two ways—firstly, the significance of the thymic changes and, secondly, the result of removing the thymus from NZB mice and from mice of other strains.

Coombs positive tests can develop in some NZB mice which do not have thymic lesions and neonatal thymectomy does not prevent, and may even precipitate, Coombs conversion. It would seem, therefore, that pathological changes in the thymus are not an essential pre-requisite for the appearance of autoimmune symptoms. The hypothesis that germinal centres in the thymus are the primary production areas for clones of self-reactive cells must also be open to question.

The idea that autoimmunity is a consequence of a breakdown in the tolerance which an animal normally shows towards its own tissues was discussed. An attempt was made to resolve the apparent contradiction that although neonatal thymectomy of NZB mice may precipitate the breakdown of

autotolerance, thymectomy of neonatal or adult mice of other strains extends the period of tolerance to humoral or cellular antigens.

While the thymus must still be suspect as a participant in autoimmune reactions, the case is by no means proven and must await further experimental evidence.

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