PROGRESS IN IMMUNOLOGY II

Volume 3

BIOLOGICAL ASPECTS II

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North-Holland
American Elsevier

Progress in Immunology II

VOLUME 3

Biological Aspects II

Proceedings of the Second International Congress of Immunology
Brighton, United Kingdom, July 21-27, 1974

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1974

NORTH-HOLLAND PUBLISHING COMPANY – AMSTERDAM · OXFORD
AMERICAN ELSEVIER PUBLISHING COMPANY, INC. – NEW YORK

ISBN North-Holland:
Series: 0 7204 7033 1
Volume: 0 7204 7036 6
ISBN American Elsevier: 0 444 10755 x

Publishers:

NORTH-HOLLAND PUBLISHING COMPANY – AMSTERDAM
NORTH-HOLLAND PUBLISHING COMPANY, Ltd. – OXFORD

Sole distributors for the U.S.A. and Canada:

AMERICAN ELSEVIER PUBLISHING COMPANY, INC.
52 VANDERBILT AVENUE
NEW YORK, N.Y. 10017

PRINTED IN THE NETHERLANDS

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I. Relation between Structure and Function of Lymphoid Tissue

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INTRODUCTION

H. Cottier

We have learned in recent years that both the initiation and the full expression of immune responses rely upon cell-to-cell interactions, and increasing interest is focused on the microenvironment in which these events take place. Much of our present knowledge in this field is based on observations made in vitro. The ultimate goal, however, must be a better understanding of the interplay of cells, other tissue components, and humoral factors as it may occur in vivo.

It has become apparent that the lymphoreticular system comprises highly organized tissues with various compartments showing distinct structural and functional characteristics (1). Some of the tissue constituents are sessile, others are migrants, and both encompass subpopulations of cells showing different structural, chemical and functional features. One is impressed with the orderly spacial arrangement of these various elements, and by the maintenance of its features throughout life, despite the numerous stimuli to which these tissues are subject and against which they are able to react.

In the present symposium an attempt will be made to assess the present state of knowledge relating structure and function of lymphoid tissue. Each contribution covers a partial aspect of the problem, and it is hoped that in putting the pieces together, our understanding of the immune system may improve. Most of the progress made originates from experimental work, but human data are equally important. Pathologists have been encouraged to report human lymph node morphology in relation to immunological function (2).

Most peripheral lymphoid tissues, in particular lymph nodes, can be subdivided into compartments showing a relative predominance of either T lymphocytes, B cells, macrophages or plasma cells. It should

be emphasized, however, that even in "B cell territory" there is an admixture of T cells and vice versa. Possibilities for cell-to-cell interactions, therefore, exist at various sites within the lymphoid tissue. It will be of special interest to learn more about preferential localizations of initial events, involving different classes and subclasses of immunoglobulins in the case of humoral antibody production, and of helper functions, suppressor activities and killer cell generation in the course of developing cell-mediated immunity. Recent observations made in our laboratory indicate that cells located in the medullary portion of lymph nodes are among the first to proliferate and differentiate following primary antibody responses. Our findings also give additional support to the view that germinal centers are predominantly involved in the generation of memory B cells (3), and do not contribute markedly to antibody production elicited by the same antigen which evoked their de novo formation and expansion (4, 5). This does not contradict the finding that under certain conditions lymphoid germinal center cells can be shown to contain specific antibody (6). We have also been puzzled by the finding that aging mice form much less germinal centers in response to a primary stimulation with certain antigens than do young ones, although the amount of antibody produced is almost the same (7). Obviously, more has also to be learned concerning changing patterns of immune responses as a function of age. These and other questions will undoubtedly be answered by the five speakers we are about to hear.

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THYMIC AND BURSAL MICROENVIRONMENTS IN THE CONTEXT OF ALTERNATIVE PATHWAYS OF IMMUNOGENESIS

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SUMMARY

The evidence in this review indicates (i) that cells having T and B cell potentialities predate the development of the thymus in vertebrate phylogeny and ontogeny, and (ii) that, in ontogeny of higher vertebrates, some of the potential immunocompetent cells are able to complete their maturation in the absence of a thymus or avian bursa, suggesting the existence of alternative pathways of immunogenesis.

The relative numbers of T and B cell antecedents left in the animal after early thymectomy or bursectomy, the time allowed for lymphoid system restitution before testing, and the intervening immunologic "stress" are considered the principal variables influencing the eventual capability for immune expression by an alternate pathway.

An attempt to integrate these ideas with some facts of thymic and bursal activity leads to the proposal that the "central" lymphoid organ microenvironments may simply expand and, in some cases, allow for further maturation of diverse clones of antecedent immunocompetent precursor cells.

This clonal expansion and maturation activity is considered as supplementary to a low background of such activity going on elsewhere, possibly in fetal liver and adult bone marrow, the principal repositories of antecedent cells. Accordingly, thymic autonomy is challenged and evidence that intrathymic and intrabursal clonal expansion could be tuned in part by antigens sequestered by the respective organs and in part by a feedback inhibition emanating from the peripheral system of descendant lymphoid cells is discussed.

1. INTRODUCTION

Recent years have seen the accumulation of a diverse body of data seemingly opposed to a required participation by the thymus and the avian bursa of Fabricius in immunogenesis. On their face, these data imply that higher vertebrates could have immune developmental pathways not associated with "central" lymphoid organs. What then are these data, what might be some of their further implications, and how might "central" organs relate to such alternative pathways?

2. IS THE THYMUS OBLIGATORY?

The alternative pathway concept derives in part from sheep thymectomy experiments (for review see 20). This work shows that *in utero* thymectomy of lambs, after 60-70 days of a 150-day gestation period, does not disturb the appearance or vigor of skin allograft reactivity in later fetal life or in postnatal life. Antibody responses in lambs aged 3 or more months are likewise unaffected, but tuberculin reactivity is slightly reduced, and lymphocyte transfer reactivity greatly reduced. This configuration is noteworthy in that the thymus is ablated before the intact fetal lamb acquires the specific competencies. Moreover, skin allografts are destroyed even if the fetal lambs are treated after thymectomy with an antilymphocyte antiserum (ALS) to eliminate existing peripheral lymphocytes (26). The grafts are destroyed as lymphocytes reappear postnatally.

Prolonged depression of peripheral lymphocyte levels seems to be the major discernable consequence of early *in utero* thymectomy of lambs (20). But recovery to normal levels is eventually reached postnatally, even by lymphocyte-free, ALS-treated, thymectomized fetuses. Morris (20) concludes that extra-thymic sites in thymusless lambs can generate cells equivalent to the so-called thymus-derived lymphocytes (i.e., T cells) by a sluggish process requiring months for complete restitution.

Usually, when early thymectomy in a species does not yield incapacities of a severity comparable to those elicited in the neonatal mouse, immunocompetent cells peripheralized before thymectomy are invoked in explanation. The sheep/ALS data in particular seem to exclude this explanation, hence the alternate pathway proposal.

3. IS THE FETAL LIVER AN EXPLANATION?

The effects of thymectomy tell us that the neonatal mouse has no alternate pathway, or has at best an ineffectual one, for the cellular competencies. How might these mouse data be reconciled with the fetal lamb data? One of the most impressive differences between these species at the time of these thymectomies is the relative amount of hepatic hematopoietic tissue, the lamb being resplendent, the mouse having only a residuum or none. If a candidate organ is sought which could generate T cell equivalents, the one that suggests itself on several counts is the developing liver.

First of all, hematopoietic liver contains numerous lymphoid cells. Second, fetal liver cells bearing the chromosomal T_6 marker, injected into irradiated adult mice, repopulate the thymus and probably then emigrate to the periphery, possibly recalling a liver to thymus to periphery migration pathway in fetal life. Third,

hematopoietic mammalian liver apparently harbors certain types of immunocompetent cells long before the thymus does. Stites, *et al.*, (27) found cells responding in mixed lymphocyte culture (MLC) in human fetal liver as early as 7.5 weeks post-conception. The thymus acquired its first phytohemagglutinin (PHA)-reactive cells at 10 weeks and its first MLC-reactive cells only at 12.5 weeks. Tyan (30) showed by means of a 2-stage transfer system *in vivo* that mouse fetal liver contains immature GVH and Ig forming precursor cells before the appearance of the thymic rudiment. He discerned the need for a thymic influence in the primary host for full development of these precursors in the final host. Stutman, *et al.*, (28) also showed the need for thymic influence in the reconstitution of neonatally thymectomized mice given fetal liver cells. Giller, *et al.*, (9) recently made stable chimeras of thymectomized, irradiated mice by treatment with allogeneic fetal liver cells combined with a few thymocytes.

These data indicate that fetal liver contains a mixture of potential and mature immunocompetent cells, some of them predating the thymic rudiment. This is noteworthy since the anatomic proximity of liver to blood returning from the placenta places hepatic immune participatory cells in a teleologically excellent position to ward off invasive maternal cells and infectious foreign cells gaining access to the fetus.

4. IS THE BURSA OBLIGATORY?

Any mature immunocompetent cells identified in prethymic fetal liver would be obvious candidates for alternate pathway participation. To date, however, the only immune participatory cells positively identified in fetal liver at a time predating the thymic rudiment are mature MLC-responsive cells in man (27) and immature GVH and Ig-responsive cells in mouse (30). These responses are all believed to be Ir gene controlled (1,15). However, which of them, if any, actually evidence alternate pathway participation?

That Ig synthesis does is indicated by results of Bryant, *et al.*, (3). Testosterone treatment of embryonating chicken eggs on the 7th day of incubation was found to completely suppress the subsequent embryonic genesis of bursal epithelium in 41 of 43 birds. The complete lack of involuted or remnant bursae, including bursal sac, plicae or duct, in these birds was confirmed by serial microscopic examinations of the cloaca. These bursaless birds nonetheless collectively formed specific agglutinins in 1 of 30 cases at 10-12 days after primary stimulation, in 6 of 26 cases at 22-23 days after primary stimulation, and in 15 of 32 cases after secondary stimulation. The titers in these sluggish responses compared favorably with intact controls. These stigmata of the humoral immune system must be ascribed to a bursa-independent pathway of immunogenesis.

The site(s) of this extrabursal mediation is not known but much extrabursal steroid damage was apparent in the nonresponders. The cloacal epithelia of these birds were particularly depleted of lymphoid cells. Thymus and bone marrow were histologically normal.

Schaffner, *et al.*, (25) have shown that the bursa efficiently takes up intracloacally deposited antigenic and inert particles and is particularly well equipped enzymatically in its epithelial tufts to degrade intracloacal bacteria before they penetrate into the follicles. These observations open the possibility that the bursa evolved as an especially efficient center for environmental antigen-driven maturation of B cell precursors. Such interpretation conforms to the bursa's teleologically excellent positioning for this purpose in its ducted communication to the cloacal lumen, and suggests, with the foregoing bursectomy experiments, that a less efficient antigen-driven maturation of B cell precursors may go on in other sites pervaded by antigens.

Antibody synthesis capability in fetal lambs predates even thymic lymphopoiesis; bacteriophage injected into lambs at 35 days of gestation provokes antiphage antibodies within 6 days (26). The thymus at this time is a purely epithelial rudiment.

5. IS THE THYMUS AUTONOMOUS?

Opinion presently holds the thymus to be largely autonomous in its relations to other lymphoid organs and to the animal's antigenic experience. Antigens given peripherally to normal young adult mammals indeed elicit in the thymus few of the histological or antibody responses initiated in responding peripheral lymphoid organs. This lack of marked immune reactivity within the thymic parenchyma has been attributed to both the immaturity of its lymphocytes and to a barrier exclusion of antigens (for review see 17). There is also, in the mouse organ grafting experiments of Metcalf (17), evidence for strong systemic homeostatic controls governing the total tissue mass of the spleen but not the thymus. The intrinsic control of thymus lymphocyte formation implicit in these observations is modified hormonally, by adrenocortical steroids in stress and by gonadal steroids in the age-involuting organ.

This autonomy of the thymus conforms to its present status as a central lymphoid organ wherein lymphocyte differentiation, under intrinsic and hormonal controls, yields immunocompetent cells whose antigen receptor configurations are determined by genetic rather than environmental factors. Autonomous differentiation of lymphocytes may be a major determinant of thymic activity. But, if the thymus is not obligatory for immunogenesis, then it is reasonable that the antigenic environment, and a homeostatic relationship to peripheral lymphoid cells, actually play more significant roles in thymic activities than commonly supposed.