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Cell Growth and Gene Rearrangement Signals during the Development of T Lymphocytes within the Thymus

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Cell growth and gene rearrangement signals during the development of T lymphocytes within the thymus

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The thymus provides signals that control the proliferation and differentiation of T lymphocytes and select the repertoire of T-cell specificities. Antibodies to CD3 molecules inhibit full rearrangement of T-cell receptor β chain genes in organ cultures of early embryo mouse thymus. Whether this effect is mediated through γδCD3 expressing cells, which are present in small numbers at this stage, or through low amounts of CD3 on αβ precursor cells is unclear. A requirement for special gene rearrangement signals within the thymus is supported also by the observations that growth factors such as IL-2 and IL-4, although stimulating proliferation of precursor cells removed from the thymus, do not induce full T-cell receptor gene rearrangements.

Recent studies show that newly formed thymic lymphocytes expressing αβCD3 receptors are targets for negative selection (deletion) as a means of removing autoreactive cells. Signalling to immature thymocytes via the αβCD3 complex induces the activation of endogenous endonucleases that cleave DNA into oligonucleosomal fragments. We suggest that the activation of this mechanism is the means by which autoreactive cells are removed.

Introduction

The thymus is crucial for providing signals that control the proliferation and differentiation of T cells. Abnormalities of thymic development in man (Lischner *et al.* 1967) or in mice (Pantelouris 1971) may result in severe immunodeficiency, a consequence of the paucity of mature, functional T lymphocytes. In addition, thymectomy of newborn mice produces an immune deficiency (Miller & Osoba 1967) that is now known to be due to a failure of T-cell production.

The question arises as to whether any T cells can be produced without the thymus. Recent studies on nude mice, which lack a lymphoid thymus, show that some T cells are produced in old animals (MacDonald *et al.* 1987). However, these cells are the products of a very small number of T-cell receptor gene rearrangements and so the repertoire of specificities for antigens is limited. Clearly, T-cell production in the absence of the thymus is extremely inefficient.

The thymic milieu

T cells mature in a complex environment of thymic stromal cells. Some of the major stromal components are illustrated in figure 1. The thymus consists of lobules each of which is divisible into outer cortex, characterized by tightly packed lymphocytes, and inner medulla where a lower density of lymphocytes is found. Epithelial cells, derived from the embryonic pharyngeal pouches, are present throughout cortex and medulla. They are connected by a network of cytoplasmic processes that envelop the developing thymocytes.

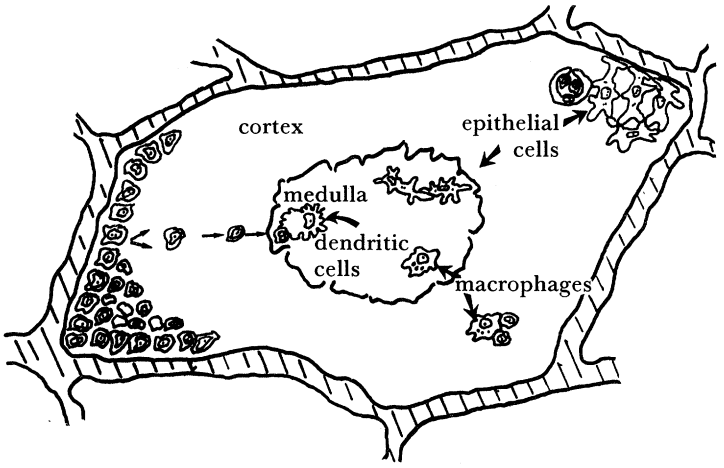

FIGURE 1. Epithelial cells and macrophages are present throughout the thymic cortex and medulla. Dendritic cells are limited to the medulla. Lymphocytes proliferate in the cortex and as they mature pass into the medulla. During the maturation pathway, lymphocytes are closely associated with the stromal cells.

Macrophages form another major stromal component and are scattered throughout the cortex and medulla. Dendritic cells, which can present some antigens very efficiently, are scarce in the cortex, but form a profuse network throughout the medulla (Barclay & Mayrhofer 1981). All of these stromal cell types are closely associated with developing lymphocytes and are likely to be involved in the maturation of the latter. Thymic epithelial cells and dendritic cells express major histocompatibility antigens that might play a role in selection of the T-cell repertoire.

T-CELL RECEPTOR GENE REARRANGEMENTS

Thymic lymphocytes are derived from stem cells that migrate into the thymic stroma in early ontogeny (Moore & Owen 1967). However, thymic lymphopoiesis is not self-sustaining and cell production is maintained by successive waves of stem cells that colonize the thymus during later foetal and adult life (Jotereau *et al.* 1987). These cells originate in sites of general haemopoiesis such as foetal liver and adult bone marrow and migrate to the thymus through the bloodstream. In experiments in which we colonized an embryonic thymus lobe with a single micromanipulated stem cell, we were able to show that the single cell could generate lymphocytes displaying multiple T-cell receptor β chain gene rearrangements (Williams *et al.* 1986). These results provide support for the notion that β chain gene rearrangements are initiated after stem cells have entered the thymic stroma. Figure 2 shows a general scheme of gene rearrangements, α chain gene rearrangements are known to follow those of the β chain and so presumably occur intrathymically as well.

Interestingly, rearrangements of γ and δ chain T-cell receptor genes probably precede those of the β chain and lead to the early appearance of a small percentage of γδCD3-expressing cells in the 15-day mouse embryo thymus (Pardoll *et al.* 1987). The time of appearance of these cells coincides with a changing pattern of gene rearrangement in the αβ cell lineage; γδ and αβ cells are thought to form separate lineages. The αβ precursor cells switch from making D–J to making full V–D–J rearrangements and we sought to test the idea that γδ cells might provide signals for this switch by doing experiments in which we attempted to block γδCD3 cell

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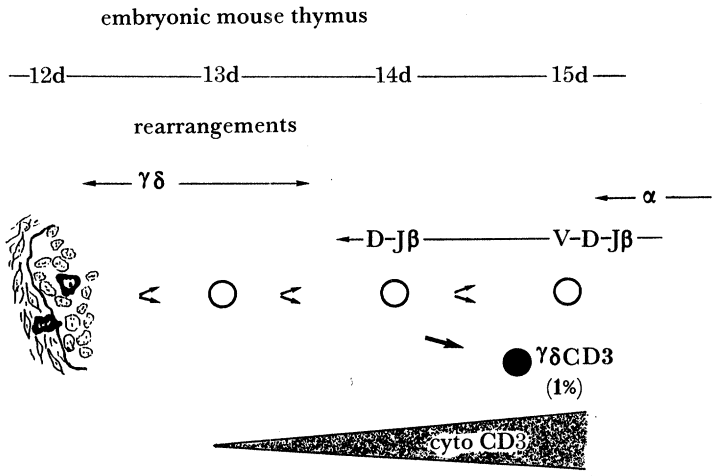

FIGURE 2. After their migration into the thymus, stem cells proliferate and their progeny undergo a series of T cell receptor gene rearrangements. These rearrangements lead to the production of γδCD3 expressing cells by the fifteenth day of gestation. However, CD3 molecules can be detected in the cytoplasm of other cells which, presumably, belong to the αβ lineage.

function by adding anti-CD3 antibodies to organ cultures of 14-day mouse embryo thymus. We found that these antibodies, but not antibodies to other cell membrane molecules, do block full V–D–J rearrangement (Owen *et al.* 1988).

The block in β chain rearrangement does not limit cell proliferation or, indeed, phenotypic differentiation. Figure 3 shows the results of experiments in which blocked cells were examined for CD4 and CD8 expression. Both molecules can be detected, although the proportions of cells that are double positive ($CD4^+$ $CD8^+$) and single positive ($CD4^+$ or $CD8^+$) differ from those found in untreated cultures.

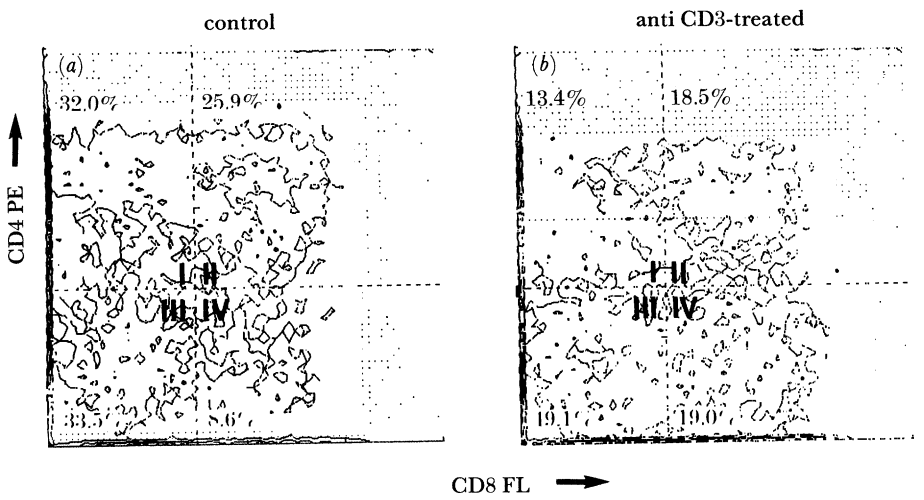

FIGURE 3. Fluorescence activated cell sorter (FACS) analysis of cells derived from organ cultures of 14-day mouse embryo thymus, some of which were treated with anti CD3 antibodies as described in Owen *et al.* (1988). Two-colour analysis was performed for CD4 expression (by using anti-CD4 antibodies conjugated to phycoerythrin, CD4PE) and CD8 expression (by using anti-CD8 antibodies labelled by fluorescein CD8FL). Box I contains cells that are $CD4^+$ only; box II contains cells that are $CD4^+$ $CD8^+$; box III, cells that are $CD4^-$ $CD8^-$, and Box IV, cells that are $CD8^+$ only. All phenotypes can be detected in anti-CD3 treated cultures despite an inhibition of T-cell receptor β chain rearrangement.

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The mechanisms by which antibodies to CD3 molecules block β chain gene rearrangements are uncertain. Although γδ cells are the only cell type expressing high levels of surface CD3 at this stage of development, CD3 molecules can be detected in the cytoplasm of other cells. The question remains as to whether the effect of the anti CD3 antibody is mediated via altered signal production in the γδ lineage or by a direct effect on low levels of CD3 molecules expressed on αβ precursor cells themselves.

Cell growth signals within the thymus

The early phases of thymic development are characterized by intense proliferation within the precursor cell compartment. The discovery of cell membrane receptors for the growth factor interleukin-2 (IL-2) on maturing thymus lymphocytes has aroused considerable interest in the possibility that this system might be involved in thymopoiesis (Ceredig *et al.* 1985; Raulet 1985). However, doubts about the functional significance of these receptors have centred on the fact that the antibodies used in the detection system identify only one of the receptor chains and so the receptors might be of the single chain, low affinity type incapable of transmitting proliferation signals (Lowenthal *et al.* 1986). On the other hand, thymic precursor cells isolated *in vitro* from the early embryonic thymus do proliferate in response to IL-2, although they do not undergo full T-cell receptor gene rearrangements (Pelkonen *et al.* 1987). Proliferation in thymus organ cultures (Jenkinson *et al.* 1987) and differentiation within the thymus *in vivo* (Tentori *et al.* 1988) are affected by antibodies to IL-2 receptors, providing further evidence for the functional importance of this system.

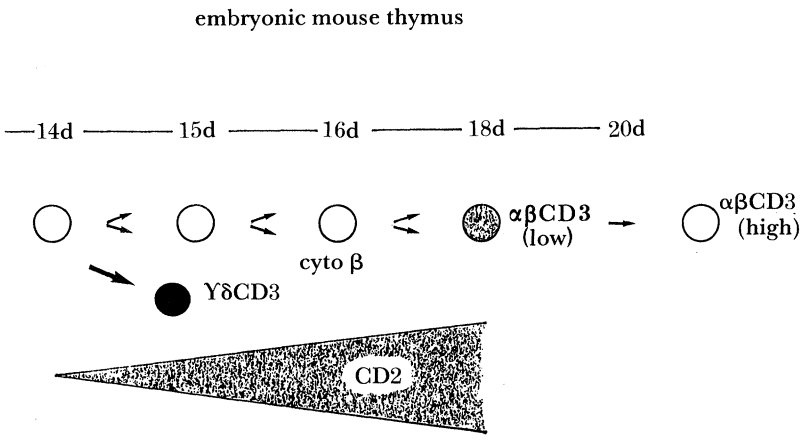

Figure 4. Later stages of thymocyte development in the mouse embryo result in the appearance of cells that express low levels of αβCD3 T-cell receptor molecules (after a stage of cytoplasmic β chain expression only). The cells with low-level expression are situated in the thymic cortex, whereas cells with high-level receptor expression are found in the thymic medulla. CD2 expression is first detected at 14 days gestation and reaches adult levels of expression by 18 days gestation.

A role for other growth factors in thymocyte proliferation is indicated in *in situ* hybridization studies where IL-4 mRNA has been detected in thymus lymphocytes (Sideras *et al.* 1988). The involvement of IL-1 at early stages of thymopoiesis is suggested by studies that have shown the importance of IL-1 derived from phagocytic cells in the induction of proliferation in stem cells (Papiernik *et al.* 1987). The signals that initiate growth factor production are poorly

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understood. It has been suggested that the CD2 molecule on immature thymocytes (figure 4) after interaction with its complementary molecule LFA-3 on thymic epithelial cells leads to activation of the IL-2 growth system (Alcoten *et al.* 1987). However, we have shown that inhibition of CD2 expression by anti-CD2 antibodies does not suppress thymocyte proliferation and differentiation (Kyewski *et al.* 1989).

The relative importance of the growth factors that have been identified within the thymus remains unclear. Further work is required into their sites of production and the stages at which they produce their effects.

Cell selection within the thymus

Newly formed thymic lymphocytes expressing αβCD3 receptors leave cell cycle (Parkin *et al.* 1988) and become targets for negative selection as a means of removing autoreactive cells and for positive selection that biases the receptor repertoire to the recognition of foreign antigens in association with self major histocompatibility antigens (reviewed in von Boehmer *et al.* (1989)). Recently, we have investigated the nature of the signals involved in the negative selection process. We found that antibodies directed to the CD3 component of the αβCD3 receptor induce apoptosis (programmed cell death) in immature thymus cells within thymus organ cultures (Smith *et al.* 1989). Apoptosis is characterized by the activation of endogenous endonucleases that cleave deoxyribonucleic acid (DNA) into oligonucleosomal fragments as a result of which nuclei become condensed and affected cells are phagocytosed by macrophages. Our results suggest that activation of the T-cell receptor complex in immature thymocytes results in cell death by this process in contrast to the induction of cell proliferation seen when the receptors of mature T cells are engaged.

Negative selection by antigens has been most clearly demonstrated in the case of superantigens, molecules that interact with the receptors of many T cells by virtue of their properties of binding to variable regions of β chains (White *et al.* 1989). Some of these antigens are bacterial products and we have found that one of them, staphylococcal enterotoxin B, which is known to delete Vβ8 and Vβ3 cells *in vivo* (White *et al.* 1989), also deletes Vβ8 immature thymocytes in thymus organ cultures. In addition, we find that this effect is associated with the appearance of oligonucleosomal fragments characteristic of apoptosis (our unpublished data). These observations provide further support for the notion that immature T cells pass through a developmental 'window' within the thymus where signalling via the T-cell receptor produces cell deletion by apoptosis.

It is obvious that experimental systems are now available to allow an analysis of some of the key events in T-cell maturation.

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