Histochemistry of Receptors

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5.3 Ontogeny of T-cell surface molecules and receptors in the thymus

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Introduction

The thymus plays a fundamental role in the maturation of immunocompetent T-lymphocytes that express receptors (T-cell receptor: TCR) recognizing and responding to foreign antigens in association with self-MHC molecules and discriminating between «self» antigens and «non self» or foreign antigens.

Intrathymic T-cell development can be divided into three stages (for review: Fowlkes and Pardoll 1989): (1) early genetic events leading to TCR expression, (2) cellular selection, and (3) acquisition of mature effector function.

Given the extreme heterogeneity prevalent among adult thymocytes, dissection of these developmental events has proved extremely difficult. One approach to studying this process has been to analyze the fetal thymic ontogeny. Indeed early fetal thymuses are highly enriched in T-cell precursors and fetal thymocytes proceed in a relatively synchronized wave: their ontogeny thus provides clues to the understanding of the differentiation sequences involved.

In this place, we will describe for the murine thymus the ontogeny of surface molecules involved in antigen recognition. We will then review their crucial role in the steps of intrathymic selection. In both cases, we will discuss the role of the thymic stroma in delivering the signals required for the full development of T-cells.

Intrathymic acquisition of surface molecules involved in antigen recognition

Surface molecules involved in antigen recognition

Mature T-lymphocytes use surface receptors to recognize and respond to foreign antigens in association with products of the major histocompatibility complex (MHC) (Zinkernagel and Doherty 1975). For the majority of T-cells (> 95%), this receptor consists of a clonally distributed disulfide-linked αβ heterodimeric T-cell receptor (TCRαβ) (Meuer et al. 1983) whose subunits are encoded by sets of rearranging gene segments (Malissen et al. 1984; Winoto et al. 1985). The TCRαβ heterodimer is noncovalently associated with an invariant membrane complex termed CD3, which is involved in signal transduction iniated when TCRαβ is engaged by the

antigen-MHC complex (CLEVERS et al. 1988). Virtually all mature αβ-bearing T-cells express either CD4 or CD8 «accessory molecules» which are involved in class II and class I recognition respectively and coparticipate with TcRαβ in antigen-specific T-cell responses (MUSTELIN and ALTMAN 1989). The remaining 5% of T-cells express a second heterodimeric T-cell receptor (termed TCRγδ) which is also associated with a CD3 complex (KRANGEL et al. 1987). The nature of the TCRγδ ligand is presently unknown.

Ontogenic studies: lineage relations of the major thymocyte subpopulations

In the embryo, the ectoderm of the third brachial cleft and the endoderm of the third pharyngeal pouch fuse together to provide an epithelial thymic primordium (AUERBACH 1961) which is colonized by lymphoid precursors between day 11 and day 12 of gestation (MOORE and OWEN 1967). These precursors express neither CD4, nor Cd8, nor surface T-cell receptors (BLUESTONE et al. 1987). This CD4-8-3- population defines the earliest intrathymic developmental stage. The majority of TCR gene rearrangements occur within this population that is in a stage of rapid division.

Complete rearrangements capable of encoding productive cell surface structures occur later – TCRγδ is first detected on day 14 to 15 of gestation, and precedes the expression of TCRαβ by 2 to 3 days. Subsequent to day 17, there is a progressive increase in the proportion of cells that express TCRαβ until, by day 20 (time of birth), TCRαβ-bearing cells represent the major CD3+ thymocyte population (PARDOLL et al. 1987).

The accessory molecules CD4 and CD8 are first expressed on days 15 to 16 of development (CEREDIG et al. 1983). Initially, there is a short period in which a fraction of thymocytes express only CD8 (KISIELOW et al. 1984). By day 17 of ontogeny, virtually all the accessory molecules bearing thymocytes express both CD4 and CD8. This CD4+8+ subset increases in absolute number and proportion until it represents 75–80% of the total thymocyte population a few days after birth. Between days 17 and 18 of gestation, the first CD4+8– thymocytes appear, followed shortly thereafter by an increase in the number of CD4–8+ thymocytes.

Within the developing thymus, TCRαβ is almost always coexpressed with accessory molecules (PARDOLL et al. 1987), the one exception being a population of CD4-8-TCRαβ+ thymocytes detected 2 to 5 days after birth. TCRγδ is expressed exclusively on DN thymocytes (CRISPE et al. 1987).

The transient CD4–8+ population present on day 16 is CD3– (TCR–); it appears to represent thymocytes on their way to becoming CD4+8+. The first CD4+8+ which appear between days 16 and 17 are also CD3-. By day 18, 5% of CD4+8+ thymocytes have begun to express low levels of TCRαβ. In contrast, the CD4+8– and the CD–8+ thymocytes which appear on day 18 and 19 express high levels of TCRαβ. They represent respectively precursors of MHC-restricted T-helper and cytotoxic cells (CEREDIG et al. 1983; MARRACK et al. 1988).

There are thus a total of 8 phenotypically distinct subsets of thymocytes defined by the four surface molecules CD4, CD8, TCRαβ, and TCRγδ. All of these subsets can also be found in the adult thymus (BOYD and HUGO 1991).

Cell-cell interactions in early thymic development

Colonization of the thymic primordium by stem cells as well as ensuing proliferation and gene rearrangements are controlled by the thymic stroma.

Thymic stem cells migrate from blood vessels into the thymic stroma, probably in response to liberation of thymic substances from the thymic epithelium (DARGEMONT et al. 1989).

Several growth factors produced by lymphocytes and stromal cells act to regulate subsequent thymocyte development. Some of them, such as IL-1, IL-2, IL-4, IL-6 and IL-7, seem to play a central role in precursor proliferation and differentiation (CARDING et al. 1991); such others as TNF-α and IFN-γ are thought most likely to exert some influences upon the properties of thymic epithelial cells that control several steps of thymocyte maturation (DEFRESNE et al. 1990).

However, these growth factors alone are not sufficient to trigger the full program of differentiation in thymocytes. For example, rearrangements of TCR genes may require direct cell-cell interactions (OWEN et al. 1988).

Cellular selection within the thymus

Mechanisms of intrathymic selection

The completion of extensive gene rearrangements in precursors cells results in the generation of large numbers of thymocytes expressing CD4, CD8 and low levels of αβCD3 molecules; this pool of thymocytes express the whole spectrum of surface TCRs that the germ line is capable of encoding. It will be subjected to intrathymic selection. There are two stages of selection: positive and negative (VON BOEHMER et al. 1989; NIKOLIC-ZUGIC 1991). They probably occur as separate events resulting from direct cell-cell recognition. Self-peptides, bound to MHC class I and class II molecules on the surface of a selecting cell, are recognized by TCR, CD8 and CD4 molecules of the thymocyte (NIKOLIC-ZUGIC and BEVAN 1990). Positive selection favors the development of T cells capable of responding to foreign antigens in association with self-MHC molecules; negative selection induces the death of self-reactive clones. The set of TCRs that emerge constitutes what has been termed the T-cell repertoire.

During positive selection, the TCR presumably recognizes a complex of an MHC plus self peptide expressed on a thymic stromal cell in the same way that it recognizes MHC plus foreign peptides. In the absence of positive selection there is an accumulation of CD4+8+3+ cells, presumably followed by clearance of these thymocytes by programmed cell death (apoptosis) (SHORTMAN et al. 1991). Apoptosis results from activation of endonuclease within the cell, leading to DNA fragmentation into oligonucleosal fragments (MC CONKEY et al. 1990). The apoptotic cells are rapidly removed by the phagocytic action of macrophages (INABA et al. 1989).

Negative selection also involves interactions between TCR, CD8 and CD4 molecules and MHC (class I or II) – self-peptide complexes which results in signaling of maturing lymphocytes via the αβCD3 receptor, leading to apoptosis (KAPPLER et al. 1987; KISIELOW et al. 1988). Negative selection thus involves the activated destruction of autoreactive cells, which are also rapidly removed by macrophages.

Thus two types of programmed cell death occur during the stages of selection. During negative selection, cells whose TCR react too efficiently with «self structures» and are potentially self-

reactive are removed. The other type is death by default: expression of a defective TCR or one inappropriate for binding the MHC structures present in the thymus disqualifies a thymocyte from full maturation and results in death. This raises a major question: how can an unchanged receptor induce different biological responses during T-cell development? This question is far from being resolved, but recent observations made during ontogeny, or in chimeric and transgenic mice, allow a theory to be proposed (FINKEL et al. 1991).

The susceptibility of thymocytes to negative selection is controlled primarily by the nature of the coupling between the αβTCR and the CD3 complex: a window exists in ontogeny from about day 16–17 of gestation, when all immature αβ+thymocytes are resistant to deletion by ligation of αβ and not to deletion by ligation of CD3. These cells are protected from elimination by TCR-mediated cell death because their αβTCR is in some way functionally uncoupled from its associated CD3 complex. Although resistant to TCR-mediated cell death, these cells are susceptible to in situ cell death – unless positively selected as a consequence of αβTCR interactions with MHC, which may directly activate a tyrosine kinase (VEILLETTE et al. 1989) or provide a sufficiently stable interaction of CD4/CD8 with class II/class I to initiate signal transduction via these molecules. The resultant tyrosine phosphorylation promotes viability, allowing expression of a maturation program or triggering maturation of the cell into a deletion (negative selection)-sensitive stage.

Cell-cell interactions during intrathymic selection

Positive and negative selection result from direct cell-cell recognition between CD4+8+3+ thymocytes, on the one hand, and thymic stromal cells expressing MHC molecules, on the other.

The nature of selecting stromal cells is still a matter of debate. MHC antigens are expressed on several thymic stromal cell types:

- cortical epithelial cells (VAN EWIJK 1991) and particularly thymic nurse cells (TNC) which are in fact lymphoepithelial complexes where thymocytes are individually enveloped by an epithelial cell membrane forming a «caveol» (WEKERLE and KETELSEN 1980; DEFRESNE et al. 1986, 1990);
- medullary epithelial cells (VAN EWIJK 1991);
- interdigitating cells (IDC), located in the medulla and at the cortico-medullary junction and forming «rosetting» complexes with thymocytes (KYEWSKI et al. 1982).

Cortical thymic macrophages are in general class II negative, whereas medullary thymocytes and macrophages express MHC antigens only at low levels (KYEWSKI et al. 1982; VAN EWIJK 1991).

It has been shown that in the microenvironment of TNC and IDC thymocytes receive an obligatory differentiation signal via the TCR during normal development (KYEWSKI et al. 1989).

TNC are good candidates for mediating positive selection. The lymphoepithelial interactions observed within these complexes are partially mediated by MHC class II molecules (DEFRESNE et al. 1990). TNC appear at day 17 of gestation (KYEWSKI et al. 1986) and thymocytes capable of forming TNC are most abundant at days 16–17 during embryogenesis (DEFRESNE et al. 1990), a stage when CD4 and CD8 appear and when TCR starts to be detected on thymocytes resistant to deletion (FINKEL et al. 1991). These complexes contain thymocytes which are able to induce a graft-versus-host reaction (PENNINGER et al. 1990).

Negative selection seems to involve interdigitating cells (SHORTMAN and VREMEC 1991) and

probably also intrathymic B lymphocytes (Mazda et al. 1991). Interdigitating cells have been shown to form «rosettes» with a thymocyte population believed to be the immediate precursors of mature cells (Brelinska et al. 1988; Shortman and Vremec 1991) and are known to be efficient at foreign peptides presentation (Fink et al. 1984), since they provide the co-stimulatory signals necessary for cell activation in mature T-cells and for programmed cell death in thymocytes. However, the possibility that thymic epithelial cells participate in negative selection cannot be ruled out – indeed they are able to present self antigens in vivo (Lorenz and Allen 1989).

MHC class II negative macrophage seem to play a role in both positive and negative selection by phagocyting the apoptotic cells (Inaba et al. 1989).

Conclusions

This review represents a summary of events for which there now exists a reasonable consensus based on multiple experimental approaches. In particular, immunohistochemical and molecular biological studies of the intrathymic acquisition of T-cell surface receptors and molecules involved in antigen recognition have elucidated the lineage relations of the major thymocyte populations. The use of monoclonal antibodies against these receptors, also studies in chimeric and transgenic mice and the isolation of lymphostromal complexes have allowed progress to be made in the understanding of the stages of intrathymic selection.

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