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Thymocyte rosettes: Multicellular complexes of lymphocytes and bone marrow-derived stromal cells in the mouse thymus

(T-cell differentiation/intrathymic cell–cell interaction/positive selection)

B. A. Kyewski*, R. V. Rouse†, and H. S. Kaplan*

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Contributed by Henry S. Kaplan, June 1, 1982

ABSTRACT We describe the isolation and purification of multicellular complexes composed of lymphocytes and bone marrow-derived stromal cells (“thymocyte rosettes”) from the mouse thymus. These rosettes are the structural in vitro correlate of in vivo associations between lymphoblasts and I-A/E negative macrophages or medullary I-A/E positive dendritic-like cells. Both types of rosettes are preformed in vivo. The rosette-associated thymocytes display a surface antigen phenotype typical of immature thymocytes. In radiation chimeras, replacement of host thymocytes by injected bone marrow cells follows a regular pattern: donor type T cells appear first at day 11 as clusters around I-A negative macrophages and approximately 2 days later as similar clusters associated with either I-A positive cortical epithelial cells or I-A positive medullary dendritic cells. These data suggest (a) a defined sequence of lymphoepithelial interactions during intrathymic maturation and (b) a rapid proliferation of thymocytes after interaction with stromal cells.

The developmental stages through which thymocytes pass during their intrathymic pathway are not yet well understood. Theoretical considerations led Jerne to propose that self-recognition of major histocompatibility complex (MHC) antigens by immature T cells may be a critical event in the selection and diversification of the T-cell repertoire (1). This hypothesis has gained considerable indirect support from recent studies in chimeric animals, in which MHC antigens expressed on thymic stromal cells have been shown to influence the self-MHC restriction pattern of T cells (2, 3). It has been difficult, however, to evaluate the precise role of stromal cells in intrathymic selection on a cellular level in view of the heterogeneity of the thymic stroma (e.g., epithelial cells, macrophages, and dendritic cells). The recent isolation of lymphoepithelial complexes [termed “thymic nurse cells” (TNC)] made it possible to purify a subset of thymocytes defined by their direct and specific association with cortical epithelial cells (4, 5). Thus, a subset of thymocytes (~1%) selectively bound to a specific stromal cell in vivo could be analyzed in vitro (6).

By extending this approach to the isolation of thymocytes defined by their specific in vivo association with other nonlymphoid cells, we have succeeded in identifying multicellular complexes (“thymocyte rosettes”) composed of thymocytes and bone marrow-derived (nepithelial) stromal cells of the mouse thymus. We report here on their morphology, phenotypic characterization, and kinetics of regeneration. These rosettes define a microenvironment for direct intrathymic cell–cell interactions which is structurally and possibly functionally distinct from the previously described lymphoepithelial complexes.

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MATERIALS AND METHODS.

Animals. C57BL/Ka (Thy 1.2), congenic C57BL/Ka (Thy 1.1), C3H, and (C5H × C57BL/F1) mice were obtained from the inbred colony of the Radiobiology Research Division, Stanford University School of Medicine. Female mice 4–6 weeks of age were used, unless otherwise indicated.

Reagents. The following monoclonal antibodies and reagents were used: fluorescein isothiocyanate (FITC) conjugated anti-Thy 1.2 (Becton Dickinson); biotin-conjugated anti-Thy 1.1 (clone 19V E12, see ref. 6); FITC-conjugated anti-I-A<sup>b</sup> (clone PB 107 kindly provided by J. Sprent, Philadelphia); anti-I-E (clone 14-4-4 kindly donated by D. Sachs, National Institutes of Health); biotin-conjugated I-A<sup>b</sup> (clone 3.6), anti-Lyt 1 (clone 53-7.3), anti-Lyt 2.3 (clone 53-6.7), and anti-H-2 K<sup>b</sup> (clone 11.4) from the Salk Institute (La Jolla, CA); FITC- and tetramethylrhodamine isothiocyanate (TRITC)-conjugated zymosan (kindly donated by H. Wekerle, Republic of Germany); FITC-conjugated peanut agglutinin (PNA) and TRITC-avidin (Vector Laboratories); FITC-F(ab')<sub>2</sub> anti-mouse IgG (Cappel); collagenase IV (Millipore); dispase/collagenase (Boehringer); cytochalasin B, sodium azide, and DNase I (Sigma).

Thymocyte Rosette and TNC Isolation. Individual thymuses were removed, trimmed free of connective tissue, pooled, minced with scissors, and incubated in 0.5 ml of RPMI-1640 medium buffered with 25 mM Hepes (pH 7.3) for 10 min at 25°C under gentle agitation to remove most of the free thymocytes. The tissue fragments were then digested with collagenase IV (0.5 mg/ml in the same medium; 0.5 ml per thymus) at 25°C for two successive 15-min periods. The remaining tissue fragments were digested with dispase/collagenase (0.5 mg/ml in phosphate-buffered saline supplemented with DNase I at 4 μg/ml at 37°C for three or four 20-min periods, with increased agitation until the thymic tissue was completely digested. After each period, the suspended cells were separated and the remaining tissue fragments were reincubated with fresh enzyme. The collagenase fractions (containing the thymocyte rosettes) and the dispase/collagenase fractions (containing TNC) were pooled separately. Rosettes and TNC were further enriched by sequential sedimentation at unit gravity on fetal calf serum gradients as described (5). Rosettes were kept at 4°C and pelleted for 1–2 min, at 100 × g during in vitro handling. Cortisone-resistant thymocytes were obtained 48 hr after intraperitoneal injection of 5 mg of hydrocortisone succinate (Upjohn) per animal.

Fluorescence Labeling and Analysis. The staining protocol for free and intra-TNC thymocytes has been described (6). Intra-TNC rosettes were fixed in 1% paraformaldehyde for 10 min at 4°C before staining. Rosette-associated T cells were dissociated in 0.25 M Tris (pH 7.4) containing 0.1% sodium azide for 10 min on ice, pelleted, and stained with anti-Thy 1.2 and anti-TCR γδ conjugates followed by phycoerythrin-conjugated sheep antimouse IgG as a second layer.

Abbreviations: MHC, major histocompatibility complex; TNC, thymic nurse cells; FITC, fluorescein isothiocyanate; TRITC, tetramethylrhodamine isothiocyanate; PNA, peanut agglutinin; BM, bone marrow.
from the stromal cells before staining by preincubation in 0.1% sodium azide for 10 min at 37°C. Phagocytosis was measured by uptake of labeled zymosan as described (5). Stained cells were then analyzed in a fluorescence-activated cell sorter (EPICS, Coulter Electronics) or examined in a Zeiss microscope under epi-illumination.

Chimeras. Three types of chimeras were used: (i) lethally irradiated recipients [900 rad (1 rad = 0.01 gray), 250 kVp, Philips] were reconstituted with $1 \times 10^7$ semiallogeneic bone marrow cells pretreated with anti-Thy 1 and C; (ii) sublethally irradiated (400 rad) Thy 1.2 recipients were reconstituted with $5 \times 10^6$ untreated congenic Thy 1.1 bone marrow cells; (iii) unirradiated newborn mice (<24 hr old) were injected with 1.5 $\times 10^7$ untreated semiallogeneic bone marrow cells into the anterior facial vein.

Electron Microscopy. Rosettes were fixed in 2.5% glutaraldehyde, pelleted with agar to form a cohesive pellet, and embedded in Epon. Sections (0.05 μm) were examined by transmission electron microscopy.

RESULTS

Isolation and Phenotypic Characterization of Thymic Rosettes. A combined dissociation procedure allows the separate isolation and purification of (i) rosettes between thymocytes and nonepithelial stromal cells and (ii) lymphoepithelial complexes (TNC) (Table 1). This procedure is based on the following observations. Rosettes can be isolated after thymic digestion with collagenase/dispose (maximal yield, $4 \times 10^4$ per thymus) and collagenase (maximal yield, $2 \times 10^5$ per thymus of mice 3-5 weeks old) but not after trypsin digestion. Optimal numbers of rosettes are recovered at 25-30°C and are released from tissue fragments after short digestion intervals (two 15-min intervals) with gentle agitation. In contrast, TNC are optimally recovered with trypsin or dispose at 37°C and after prolonged dissociation (three to five 20-min intervals). Thus, one can obtain enriched rosette preparations (~70% of the rosettes of all fractions with 1-4% TNC contamination) by pooling the early collagenase fractions, and enriched TNC preparations (~90% of the TNC of all fractions) by pooling the late fractions. Rosette-associated T cells can be further enriched to ~90% purity by repetitive $1 \times g$ sedimentation as described (5).

Each intact rosette comprises 10–15 thymocytes. Intact rosettes are preserved in vitro only by working at 4°C and by minimization of shear forces during handling. Rosettes cannot be isolated in the presence of metabolic inhibitors (0.1% sodium azide), EDTA (10 mM), or cytochalasin B (10 μg/ml); and, after isolation, intact rosettes are rapidly dissociated by addition of these agents or trypsin (data not shown).

Rosettes (including I-A positive central cells) first appeared during ontogeny at day 14 of gestation, increased 10-fold between 1 and 2 weeks of age, and were still found in low numbers in 6-month-old animals.

It was mandatory to ensure that rosettes were not the result of random associations during the isolation procedure. Hence, we minced equal numbers of Thy 1.1 and Thy 1.2 thymuses in vitro, codigested the tissue fragments, and subsequently isolated rosettes and double-labeled the associated thymocytes for both Thy 1 antigens. If rosettes were the result of random associations, rosettes isolated from in vitro mixtures of phenotypically different thymocytes should be mixed. Thus, Thy 1 markers clearly segregated with individual rosettes (data not shown). The thymocytes of a given rosette were either Thy 1.1 or Thy 1.2 positive. However, mixed rosettes could be isolated from radiation chimeras reconstituted with bone marrow cells of different Thy 1 types (see below). These results strongly suggest that rosettes preexist in vivo and that their cellular composition is not altered secondarily during isolation. Similar results have been found for TNC (6).

Comparative flow cytofluorometric analysis of highly enriched (>90%) rosette-derived lymphocytes revealed a homogeneous surface antigen phenotype. In comparison with unselected and cortisone-resistant thymocytes, >90% of the rosette-associated T cells expressed high-density Thy 1, low-density Lyt 1, Lyt 2, 3, low-density H-2 Kk (in C3H mice) antigens and high-density PNA receptors (Fig. 1). Rosette-bound T cells were radiation- and cortisone-sensitive and consistently larger than unselected thymocytes (data not shown). Thus, they displayed the phenotype typical of immature thymocytes (7–9).

The nonlymphoid cells central to the rosettes fell into two subpopulations: (a) approximately 45–50% expressed high amounts of I-A/E antigens and were nonphagocytic and only weakly adherent to glass; (b) 50–55% of the stromal cells expressed little or no I-A/E antigens (as assessed by fluorescence microscopy) and were highly phagocytic and readily adherent. Thus, 1 (or 20) hr after in vitro cultivation of purified rosettes on glass cover slips, 90% (or 99%) of the adherent cells were highly phagocytic and I-A negative and displayed the typical morphology of macrophages, whereas 10% (or 1%) were strongly I-A/E positive, weakly if at all phagocytic, and resembled the dendritic cells described by Steinman et al. (10). The majority (~90%) of the I-A-positive stromal cells were recovered in the nonadherent population. These rosettes have been reproducibly found in neonatal (day 3) and young adult

<p>| Table 1. Isolation of &quot;thymocyte rosettes&quot; and TNC by differential dissociation |
|---------------------------------|----------------|-----------------|-----------------|-----------------|
| <strong>Dissociation procedure</strong>       | <strong>Recovery, no. x 10^-6 per 24 thymuses</strong> |</p>
<table>
<thead>
<tr>
<th>Step</th>
<th>Interval, min</th>
<th>Temp, °C</th>
<th>Enzyme</th>
<th>Unselected thymocytes</th>
<th>Rosettes</th>
<th>TNC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>25</td>
<td>None</td>
<td>1,690</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>25</td>
<td>Collagenase</td>
<td>465</td>
<td>1.2</td>
<td>0.06</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>25</td>
<td>Collagenase</td>
<td>95</td>
<td>0.53</td>
<td>0.026</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>37</td>
<td>Disparse/</td>
<td>97</td>
<td>0.32</td>
<td>0.02</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>37</td>
<td>collagenase</td>
<td>100</td>
<td>0.18</td>
<td>0.15</td>
</tr>
<tr>
<td>6</td>
<td>20</td>
<td>37</td>
<td>Total recovery, no. x 10^-6 per thymus</td>
<td>217</td>
<td>&lt;0.001</td>
<td>0.79</td>
</tr>
</tbody>
</table>

Thymuses of female C57BL/Ka mice 4 weeks of age were dissociated according to the scheme indicated. The yields represent a typical experiment.
Figure 1. Surface antigen phenotype of rosette-associated T cells. Highly purified rosette-associated T cells (curve A) and cortisone-resistant thymocytes (curve B) of C57BL mice were stained for PNA receptors, H-2Kk, Lyt 1, and Lyt 2,3 antigens, and 20,000 cells per sample were analyzed by flow cytofluorometry.

C57BL, C3H, and (C3H × C57BL)F1 mice. The composition and yield of the rosettes are unique to the thymus and could not be found in similar preparations of lymph nodes, spleen, and bone marrow. The heterogeneity of the center cells in the rosettes has been confirmed by transmission electron microscopy. They range from cells with signs of intense phagocytic activity to cells with very little evidence of such activity. The former contain many lysosomes, moderate numbers of mitochondria, and few to moderate numbers of cytoplasmic processes. The latter type contains only rare lysosomes and numerous long cytoplasmic processes. These two types of cells do not comprise nonoverlapping groups, however, because cells intermediate in appearance were also seen. Less than 1% of the central cells contained tonofilaments (or desmosomes), which are easily identified in epithelial cells such as TNC. Although many cells had lysosomes containing cellular debris, no evidence of phagocytosis of the surrounding lymphocytes was seen.

**Postirradiation Reconstitution of Rosette Stromal Cells.** Both macrophages and dendritic cells are of hematopoietic origin (for review, see refs. 10 and 11). In view of conflicting data as to the role of resident thymic and bone marrow-derived cells expressing I-A to T-cell precursor selection (12, 13), we analyzed the kinetics of the rosette-forming I-A-positive cells in two types of chimeras: (a) (C3H × C57BL)F1 (Thy 1.2) bone marrow cells were injected into lethally irradiated C57BL (Thy 1.1) recipients, and (b) [C3H (Thy 1.2) × C57BL (Thy 1.1)]F1 bone marrow cells were injected into unirradiated newborn C3H (Thy 1.2) animals. At various time intervals after reconstitution, both thymocytes and I-A-positive stromal cells were analyzed for the expression of host and donor phenotypes. The I-A-positive rosette-forming stromal cells were rapidly replaced by donor cells: as early as 2 weeks after lethal irradiation, 25% of all stromal rosette cells expressed donor-type I-A antigens (Table 2). Between 2 and 4 weeks after irradiation, both host- and donor-type thymocytes were present and mixed rosettes containing semiallogeneic partner cells could be isolated. The relative number of donor-type I-A-positive cells did not change significantly between 4 and 12 weeks postirradiation. Similarly, when newborn recipients were analyzed, donor-type I-A positive stromal cells appeared in the rosette fraction early after treatment (3–6 days) and persisted at about the same relative proportion despite a 10-fold increase in rosette yield between days 3 and 14. Furthermore, donor-type stromal cells were detectable much earlier than donor type thymocytes (Table 2). The kinetics of rosette formation in newborn recipients suggest that the replacement of bone marrow-derived stromal cells in the thymus is not merely a postirradiation effect but also occurs in the normal organ. It should be pointed out that, at all time points tested, the epithelial component of TNC consistently expressed only host-type MHC determinants (data not shown).

**Table 2.** Kinetics of I-A-positive bone marrow-derived thymic stromal cells

<table>
<thead>
<tr>
<th>Donor:Recipient</th>
<th>(C3H × C57BL)F1 (Thy 1.2, H-2k&lt;sup&gt;-&lt;/sup&gt;)</th>
<th>C57BL (Thy 1.1, H-2&lt;sup&gt;-&lt;/sup&gt;k) (900 rad)</th>
<th>[C3H (Thy 1.2) × C57BL (Thy 1.1)]F1 (H-2&lt;sup&gt;-&lt;/sup&gt;k&lt;sup&gt;-&lt;/sup&gt;)</th>
<th>C57BL (Thy 1.2, H-2&lt;sup&gt;-&lt;/sup&gt;k) newborns</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time post-</td>
<td>Donor-type (I-A&lt;sup&gt;-&lt;/sup&gt;)&lt;br&gt;rosettes</td>
<td>Rosette yield, per thymus</td>
<td>Free donor (Thy 1.2)&lt;br&gt;thymocytes</td>
<td>Donor-type (I-A&lt;sup&gt;-&lt;/sup&gt;)&lt;br&gt;rosettes</td>
</tr>
<tr>
<td>irradiation, wk</td>
<td>%</td>
<td>no. × 10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>2</td>
<td>25(25)</td>
<td>8.3</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>32(26)</td>
<td>20</td>
<td>&gt;99</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>35</td>
<td>22</td>
<td>&gt;99</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>28(26)</td>
<td>20</td>
<td>&gt;99</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2.7 (1)</td>
<td>3 (0.5)</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2.8 (1.7)</td>
<td>13 (19)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>6</td>
<td>98</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>4</td>
<td>76</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

For each time point, 8–10 thymuses were pooled and at least 500 rosettes or thymocytes were counted. Values are given as percentages; replicate results are shown in parentheses. ND, not done.
was delayed by about cells selected thymocytes after postirradiation (Thy 1.2) donor to (days 5-7) with this cells (days 12-14) associated with 1.1 BM $10^5$ cells thymocytes. FIG. 2. Repopulation kinetics of stromal cell-associated and unselected thymocytes after sublethal irradiation. Sublethally irradiated C57BL/Thy 1.2 mice were reconstituted with $5 \times 10^5$ congenic Thy 1.1 BM cells and, at various time intervals thereafter, the ratios of donor to host thymocytes in the indicated compartments were assessed. For each time point, six to eight thymuses were pooled and $5 \times 10^5$ to $1 \times 10^6$ thymocytes were counted.

DISCUSSION

We describe a method that allows the sequential isolation of (i) multicellular aggregates between lymphocytes and bone marrow-derived stromal cells (thymocyte rosettes), and (ii) lymphoepithelial complexes (TNC). We regard the thymocyte rosettes as the in vitro correlate of in vivo associations between thymocytes and bone marrow-derived accessory cells in the thymus. This interpretation is supported by several observations. The requirements for isolation of intact rosettes are similar to those allowing complex formation and immune recognition between T helper cells and antigen-presenting cells, between cytotoxic T cells and target cells in vitro, and between thymocytes and cortical epithelial cells in vivo (6, 14, 15). Furthermore, the cellular composition of rosettes is predetermined in vitro and not secondarily altered during isolation. In addition, the homogeneous phenotype of the rosette-associated T cells and their distinct repopulation kinetics after sublethal irradiation identify them as a separate subset. Finally, the relative abundance of rosettes during ontogeny correlates well with intrathymic differentiation of functional T cells.

The stromal cells forming the rosettes are of at least two types. About half are typical macrophages as assessed by their morphology (by light and electron microscopy), their high phagocytic activity, their ready adherence to glass, and their Fc type (data not shown). It is unlikely that these results are due to a preferential radiosensitivity of the I-A-positive dendritic cells because these stromal cells do form rosettes with host T cells when donor cells are still preferentially associated with I-A-negative stromal cells.

During their early phase of intrathymic expansion (days 12–16), donor-derived cells were not randomly distributed in the rosette and TNC compartments but appeared highly clustered in individual rosettes or TNC. The clustering (defined by more than four donor cells per aggregate) corresponded to a 50- to 100-fold enrichment of donor cells in individual complexes compared to their average frequency in the respective compartment. Thus, at day 13, when the overall frequency of donor cells in the rosette- and TNC-associated T cell subsets ranged between 0.1% and 5%, individual complexes already contained 50% or more donor-derived cells (Fig. 3).
receptor expression (data not shown). These macrophages were essentially I-A/E negative both after isolation and after short term in vitro cultivation. The second type (40–45%) has been assigned to the dendritic cell lineage (10) on the basis of their morphology, limited capacity for adherence, low phagocytic activity, and strong constitutive I-A/E antigen expression. This cellular composition was indirectly confirmed by functional studies; purified rosettes present antigen very efficiently in vitro (unpublished data). Careful analysis of the purified rosette population by electron microscopy revealed that they are essentially free of epithelial cells (except for contaminating TNC which are easily identified) as judged by the absence of tonofilaments and desmosomes in the nonlymphoid cells. Hence, the stromal cells of the early collagenase fractions comprise accessory cells of hematopoietic origin. This view has been confirmed by the results in F1 → parent chimeras. The I-A-positive rosette stromal cells were rapidly and completely replaced by donor-type marrow-derived cells after lethal irradiation, whereas the epithelial component of TNC consistently expressed only host MHC determinants. Thus, as early as 2 weeks after reconstitution, at the onset of T-cell differentiation in the regenerating thymus, donor-type thymocytes were found to associate with donor-type dendritic cells and host-type epithelial cells. It is notable that donor bone marrow-derived I-A-positive stromal cells formed rosettes as early as 3 days after injection into newborn recipients, possibly indicating that these cells have a substantial rate of physiological turnover (16). The methods used did not allow a clear assignment of macrophages to donor or host type.

The different stromal cells presumably occupy distinct compartments. TNC have been shown to reside in the outer cortex, whereas I-A-positive stromal cells of hematopoietic derivation are confined to the medulla and the corticomedullary junction (6, 17, 18). The exact in situ localization of the macrophages forming the rosettes is less well-defined because macrophages have been found in the inner cortex, the corticomedullary junction, and the medulla (18–20).

The rosette-associated thymocytes comprised a homogeneous population. No marker so far tested distinguished thymocytes selectively associated with either macrophages or dendritic cells. Thymocytes defined thus far by in vitro association with all three types of stromal cells have a surface antigen phenotype typical of immature thymocytes, despite their different intrathymic localizations (6). It might be speculated that they belong to a common precursor stage whose subsequent differentiation into different lineages is induced by the respective stromal cell.

The postirradiation kinetics demonstrate a defined sequence of lymphostromal interactions during intrathymic T-cell differentiation. Donor-derived T cells first associate with and expand around I-A-negative macrophages, presumably in the central thymus, and, after a significant delay, similar interactions occur simultaneously with resident epithelial cells in the cortex and dendritic cells in the medulla. This sequence is paralleled during ontogeny by the first detection of rosettes at day 14 and of TNC at day 17 of gestation (data not shown). Whether the observed postirradiation events accurately reflect physiological pathways (21) and whether any precursor-product relationship exists between the different compartments await further studies.

An interesting feature of the early phase of donor-cell expansion during thymic regeneration was the clustered distribution of T cells in individual rosettes (both types) and TNC. These prominent clusters could be the result either of a rapid ("clonal") proliferation of activated thymocytes after interaction with stromal cells or of accumulation due to the strategic localization of the stromal cell at a site where donor cells enter the respective compartment. We favor the former interpretation. Purified stromal cell-associated T cells were relatively enriched in cycling cells (as measured by DNA histogram profiles) and showed a significantly greater extent of spontaneous proliferation in vitro in the absence of any added antigen or mitogen, when compared with unselected thymocytes (unpublished data). All three types of intercellular communications Thus may be involved in positive expansion of immature T-cell populations. It will be important to determine whether or not these associations represent recognition of self-MHC antigens and whether proliferation is the result of antigen-driven selection of self-reactive T cell precursors, as originally proposed by Jerne (1).

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