Thymotaxin: A thymic epithelial peptide chemotactic for T-cell precursors  
(thymus/T-cell differentiation in vitro/bone marrow in vitro/stem cells)

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ABSTRACT The embryonic thymus is seeded by invading hemopoietic precursor cells that differentiate intrathymically into T lymphocytes. We have recently reported that avian thymic epithelial cells secrete chemotactic peptides, which provoke oriented migration of hemopoietic precursor cells in vitro. The established rat thymic epithelial cell line IT-45 R1 produced a polypeptide that resolves as a single band in the region of 11 kDa on NaDodSO4/polyacrylamide gels. This molecule, which we have named thymotaxin, induced a chemotactic response in a subpopulation of hemopoietic cells from juvenile rat bone marrow. Responding cells were generated by short-term coculture of rat bone marrow hemopoietic cells with mouse bone marrow stroma in a steroid-free medium. Cells selected in a chemotactic chamber have a lymphoid or blast cell morphology. The phenotype of the responding cells is Thy-1+, CD4+ and CD8+. In contrast, CD8 T-lymphocyte differentiation antigen was expressed after coculture with embryonic thymic monolayers, suggesting that the responding cells correspond to the precursors colonizing the thymus.

The thymic microenvironment plays a crucial role in the differentiation of immigrated hemopoietic precursors into T cells (1–5). Direct cell–cell interaction as well as soluble factors are involved in the establishment of the different T-cell lineages (6–10). Thymic epithelial cells of the superficial cortex and of the medulla have attracted particular interest since they produce functional thymic hormones such as thymopoietin, thymosin, or thymulin (11, 12). Besides the secretion of hormones that play important roles in the differentiation of hemopoietic precursors, thymic epithelial cells are involved in the recruitment of T-cell precursors to the thymus. Avian thymic epithelial cells, prepared from quail embryos, secrete peptides that are chemotactic for hemopoietic precursor cells (13, 14). Quail precursors can be selected by chemotactic migration in vitro toward these peptides in Boyden chambers. These quail cells subsequently still have the capacity to colonize a xenogeneic chicken thymus in vitro. When this colonized thymus is grafted back to a chicken embryo, the quail hemopoietic precursors mature into T cells. In avian embryos, cells responding to thymic chemotactic peptides are found in the bone marrow (15); early on, however, they may also originate from discrete hemopoietic foci that are dispersed in the embryo (16). To reach the thymus, these hemopoietic cells extravasate from blood vessels, traverse the perithymic mesenchyme, and finally invade the thymic basement membrane before inserting themselves between epithelial cells. The chemotactic peptides are directly involved in these events by exerting a dual function in controlling the oriented migration and by inducing a transient invasive behavior (15).

Recently, a rat thymic cell line (IT-45 R1) was established and shown to possess all the properties of epithelial cells (17). These cells show morphological and functional aspects of the superficial cortex epithelium. By immunofluorescence, thymulin as well as thymopoietin can be detected in the cytoplasm of IT-45 R1 cells (18). These cells secrete thymulin, and their conditioned medium contains this thymic hormone as a biologically active molecule. In the present study, we show that this rat thymic cell line produces chemotactic molecules comparable to the avian system. Hemopoietic precursor cells responding to the chemotactic peptides are found in the bone marrow of young rats. An in vitro culture system of bone marrow cells grown on mouse stroma using steroid-free sera allowed the enrichment of responding cells. The responding cells predominantly had a lymphoid phenotype and were devoid of T- or B-cell differentiation markers. A significant percentage of these hemopoietic cells selected by the chemotactic peptides were able to express T-cell markers when grown in contact with primary cultures of embryonic rat thymus.

MATERIALS AND METHODS

Animals. Male WAG Wistar rats and male Swiss mice, 3–4 weeks old, were routinely used for bone marrow preparation.

Hemopoietic Precursor Cells. Bone marrow cells were prepared from the femur cavity and loaded onto bovine serum albumin (Path-O-cyte-5, Miles) diluted to 28% with phosphate-buffered saline (PBS) and centrifuged at 1000 × g for 30 min. Floating cells were washed with Dulbecco’s modified Eagle’s medium (DMEM; GIBCO) and used for chemotactic migration assays.

Chemotactic Migration Assays. For migration assays, Boyden chambers (100 μl) were used as described (14). The cells were counted with a Coulter Counter ZM equipped with a channelizer 256; the counting window was 5–9 μm and aperture size was 140 μm. Approximately 5 × 105 bone marrow cells or 2–10 × 104 cultured cells were loaded in the upper compartment of Boyden chambers. Testing media were placed into the lower blind well chamber, which was separated from the cell-containing compartment by Nucleopore filters (pore size, 5 μm; Nuclepore, Pleasanton, CA). After 3 hr incubation at 37°C, the cells of the upper compartment were removed by washing five times with PBS. The chamber was then centrifuged at 100 × g to detach weakly adherent cells from the lower surface of the filter, the Nuclepore filter was discarded, and the migrated cells were

Abbreviation: IL-1, interleukin 1.

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collected from the lower compartment and suspended in 10 ml of Isoton II (Coultronics, Margency, France). Aliquots (500 μl) were assayed on the Coulter Counter.

For cytological analysis, hemopoietic cells were cytacentrifuged in a Cytospin 2 (Shandon, Cheshire, England). Centrifuged cells were incubated in concentrated May-Grünwald solution (Merck), followed by staining in 7% Giemsa solution. For determination of the cytological type, at least 200 cells per slide were analyzed.

**Cultured Hemopoietic Precursor Cells.** Short-term cultures were prepared according to Hayashi et al. (19) with the following modifications: adherent feeder cells were obtained by culture of total mouse bone marrow cells in 5 ml of Iscove’s modified Dulbecco’s medium (IMDM; GIBCO), 20% horse serum (GIBCO) at 37°C, 7.5% CO₂ in a humidified atmosphere at a density of 10⁶ cells per 5 ml per 25-cm² culture flask (Nunc). After 3 days, an additional 5 ml of fresh medium was added, and, at day 6, the medium was renewed. At day 10, the flasks were washed twice with 5 ml of IMDM and the medium was replaced by IMDM containing 20% fetal calf serum (Flow Laboratories) preabsorbed on charcoal (Norit A, Serva, Heidelberg) and Dextran K70 (Pharmacia) to remove steroid hormones, as described (19), and 1% steroid-free synthetic serum U1roser SF (IBF, Villeneuve-la-Garenne, France). Rat bone marrow cells enriched for hemopoietic precursors on 28% bovine serum albumin centrifugation gradients were then cocultured with the adherent mouse feeder layer (3 × 10⁶ cells per 7 ml per flask). Culturing for 3 days produced weakly adherent and floating cells with a diameter of 6.2 μm that were used for migration assays.

**Differentiation of Hemopoietic Cells on Thymic Tissue from Rat Embryos.** Thymuses of 14- to 15-day-old rat embryos taken just at the onset of colonization were explanted, cut into two parts, and cultured in microwell dishes (96 wells; Nunclon, Delta, Nunc) for 3 days in IMDM containing D-valine (GIBCO), 15% fetal calf serum (Flow Laboratories), ascorbic acid (50 μg/ml), reduced glutathione (100 μg/ml), insulin (10 μg/ml), and NADH (10 μg/ml) (20). After 3 days, the adherent tissue was washed and the medium was exchanged by IMDM containing 20% steroid-free fetal calf serum and 1% synthetic serum U1roser SF. Hemopoietic cells (10²) that had migrated were added to the culture. At day 3, cells were harvested and analyzed for their T-cell surface antigens.

**Immunolabeling.** Immunofluorescence was performed on cell suspensions by using the following mouse monoclonal antibodies at dilutions indicated by the supplier: Ox-6 (major histocompatibility complex class II), Ox-7 (Thy-1), Ox-8 (CD8), W3-25 (CD4), and Marm 4 (IgM), all from Serotec (Oxon, England). Fluorescein-coupled goat anti-mouse immunoglobulin (Nordic, Tilburg, the Netherlands) was used as second antibody (1:100).

**Purification of Thymic Chemotactic Peptides.** IT-45 R1 cells were cultured in DMEM/20% fetal calf serum (Flow Laboratories) in plastic roller bottles (850 cm²; Corning). When the cells reached subconfluency at a density of 1.1 × 10⁶ cells per cm², medium was exchanged for 200 ml of serum-free DMEM per roller bottle. After conditioning for 3 days, the supernatant was centrifuged, and filtered, and 1 liter was loaded at 80 ml/hr onto five Sep-Pak C₁₅ cartridges (Millipore, Waters Associates). The material was eluted from Sep-Pak by 1.5 ml of 80% acetonitrile and loaded onto a Sephadex G-50 (Pharmacia) column (1.5 × 100 cm) equilibrated with PBS and run at 10 ml/hr. Fractions (2.5 ml) were lyophilized to allow better storage, reconstituted with H₂O, diluted in DMEM to the original volume of conditioned medium, and tested for chemotactic activity on hemopoietic precursors in Boyden chambers. Aliquots were analyzed on silver-stained NaDodSO₄/polyacrylamide gels. Peptide concentrations were determined by the Bio-Rad protein assay.

**Thymocyte Activation and Cytotoxicity Used as Detection Assays for Interleukin 1 (IL-1) Activity.** Activation of thymocytes was performed as described (21). Briefly, 1.5 × 10⁶ thymocytes were cultured in 96-well plates for 48 hr in the presence of phytomethagglutinin P (1 μg/ml) (Wellcome). After 48 hr, 1 μCi of [3H]thymidine (specific activity, 1 Ci/mmol; 1 Ci = 37 GBq; Commissariat à l’Energie Atomique, Saclay, France) was added to each culture well and the cells were incubated for an additional 20 hr. Cytotoxicity mediated by IL-1 was performed on L929-a target cells that were maintained at 10⁶ cells per well. Twenty-four hours later, serial 1:3 dilutions of monocyte conditioned medium or testing medium were added to the cells with actinomycin D (1 μg/ml) (Sigma). The plates were reincubated for 20–24 hr at 37°C. Thereafter, medium was removed and the cells were stained with 0.5% crystal violet dye for 15 min. A unit of cytotoxic activity is defined by the reciprocal of the dilution that causes a 50% reduction in cell number under conditions of the assay.

**RESULTS**

**Chemotactic Migration of Hemopoietic Precursor Cells Toward Thymic Epithelial Conditioned Medium.** The most abundant source of hemopoietic precursors was the bone marrow of 3-week-old rats. Total bone marrow cells contained three major cell populations defined by their size using a Coulter Counter channelizer 256 (Fig. 1a). The first cell population, with an average diameter of 4.4–4.5 μm, contained mostly erythrocytes and erythroid precursors; the second corresponded to cells having a diameter of 5.5 μm and contained mostly cells of the lymphoid type (22); the third size class, constituted by cells with a mean diameter of 7 μm, was mainly of the myeloid type. After centrifugation on a cushion of 28% bovine serum albumin, lymphoid precursor cells and other immature cells were found in the floating layer corresponding to 10% of the total cells (Fig. 1b). All mature erythrocytes, polymorphonuclear leukocytes, granulocytes, and mastocytes were found in the pellet. When 5 × 10⁶ bone

![Fig. 1](https://example.com/fig1.png)  
**Fig. 1.** Histogram of bone marrow cells before and after in vitro migration. The diameters of rat bone marrow cells were analyzed on a Coulter Counter channelizer 256. (a) Fresh bone marrow cells of 3-week-old rats. (b) Bone marrow cells enriched for hemopoietic precursor cells on a bovine serum albumin gradient. (c) Hemopoietic precursor cells from the bovine serum albumin gradient after coculture on mouse bone marrow stroma for 3 days. Cells with a mean diameter of 6.2 μm were greatly enriched. (d) Hemopoietic precursors after bovine serum albumin gradient that migrated toward IT-45 R1 thymic epithelia conditioned medium. The two cell populations detected by window settings 1 and 2 migrated specifically. The smaller erythroid lineages also migrated toward control medium and were therefore not counted. (e) Cultured cells that migrated toward IT-45 R1 conditioned medium.
marrow cells, 10 times enriched for precursors and lymphoids on bovine serum albumin, were exposed to IT-45 R1 thymic epithelial cell-conditioned medium in Boyden chambers, 1.7 ± 0.18% of these cells migrated toward chemotactic factors present in this medium. A histogram of cells after chemotactic migration performed on the channelizer 256 showed two migrating cell populations defined by their size (Fig. 1d). These cells were further analyzed after cytocentrifugation and Giemsa–May–Grünewald staining. Large cells (8 μm on Coulter Counter) were mostly of the myeloid type but contained 23% immature blasts, and the medium sized cells (6 μm) contained cells of the lymphoid type. Using an appropriate window setting on the channelizer 256, it was possible to distinguish migration of the two major cell subpopulations. The 6-μm cell population migrated specifically toward thymic epithelial cell conditioned medium, whereas large cells also migrated toward components in the fetal calf serum (data not shown). Therefore, the migration effect of small 6-μm cells that contain lymphoids was used for subsequent studies.

Short-Term In Vitro Culture of Hemopoietic Precursors from Bone Marrow. In an attempt to further enrich hemopoietic cells that migrate specifically toward thymic factors, rat bone marrow cells after bovine serum albumin gradient centrifugation were cocultured with mouse bone marrow monolayer in the presence of 20% steroid-free fetal calf serum supplemented with 1% steroid-free synthetic serum. A cell population of intermediate cell size (diameter, 6.2 μm) appeared after 24 hr of culture and persisted for several days (Fig. 1c). Most of these cells weakly adhered to feeder cells (Fig. 2). Upon exposure to the thymic epithelial cell-derived medium, 7% of these cells migrated in Boyden chambers at day 3 of culture (Fig. 1e). Migration competent precursors are therefore 41-fold enriched under these culture conditions compared to those found in freshly prepared total bone marrow cells. Noticeably, the contaminating erythroid precursor cells disappeared from cultured cells, and cells of the lymphoid type with a diameter of 6 μm were enriched in such populations (Fig. 1c; Table 1). As much as 60% of migrated cells express the Thy-1 molecule, whereas no T-cell (CD4, CD8) or B-cell differentiation markers (IgM, major histocompatibility complex class II) were detectable on these cultured bone marrow cells, suggesting an immature phenotype.

Isolation of the Chemotactic Activity Secreted by the Thymic Epithelial Cell Line IT-45 R1. For rapid concentration, serum-free IT-45 R1 thymic epithelial cell-conditioned medium was passed through Sep-Pak C18 cartridges. The cartridges were eluted with 80% acetonitrile and aliquots were lyophilized and reconstituted to the original volume by fresh DMEM. Checkerboard analysis in Boyden chambers confirmed that cultured bone marrow cells of the lymphoid type migrated

| Table 1. Discrimination between chemotactic and chemokinetic activity of IT-45 R1 conditioned medium on lymphoid type cells |
|-----------------|-----------------|-----------------|
| Upper well      | Lower well      | Migrated lymphoid cells |
| Control medium  | Control medium  | 129 ± 51        |
| Control medium  | Conditioned medium | 358 ± 8        |
| Conditioned medium | Control medium  | 193 ± 58        |
| Conditioned medium | Conditioned medium | 109 ± 42        |

Thymic epithelial cell conditioned medium was purified on a Sep-Pak cartridge and the eluted activity was reconstituted to the original volume by DMEM. Tests were performed in Boyden chambers with 2.5 × 10⁶ cultured bone marrow cells. Migrated cells were counted and analyzed for their phenotype by May–Grünewald–Giemsa staining. Lymphoid type cells migrated chemotactically toward IT-45 R1 products. Standard deviation was given for three experiments. Phenotype of responding cells was determined by counting at least 250 cells that migrated in preparative Boyden chambers.

chemotactically toward these thymic epithelial cell products (Table 1). For further purification, cartridge eluted material was loaded onto a Sephadex G-50 column. All fractions were diluted with DMEM to the original volume of the conditioned medium and tested for chemotactic migration of cultured bone marrow cells in Boyden chambers. Chemotactic activity was found in two zones of 11 and 4 kDa (Fig. 3A). Cells that migrated toward the different column fractions were analyzed for their morphological phenotype by May–Grünewald–Giemsa staining. The migration profile for lymphoid type cells corroborated with the active fractions that have been found by counting migrated cells by the Coulter Counter (Fig. 3A). The purified 11-kDa material was much more selective than the crude conditioned medium (75 vs. 51% of migrated cells were of lymphoid type, respectively; Table 2). The purified chemotactic activity was further analyzed by NaDodSO₄/gel chromatography. Silver-staining identified the 11-kDa material as a single distinct band, which we have named thymotaxin. Full chemotactic activity was achieved by using thymotaxin at 14 ng/ml, whereas Sep-Pak

![Fig. 2. Rat hemopoietic precursor cells cocultured on mouse bone marrow stroma. Bone marrow cells of 3-week-old rats were enriched for hemopoietic precursor cells on a bovine serum albumin gradient and cocultured on mouse bone marrow stroma in steroid-free serum. Cells with an average diameter of 6.2 μm were predominant after 3 days of culture. These precursors were round shaped and adhered around stromal cells.](image-url)
eluted material at 25 \( \mu g/ml \) or undiluted conditioned medium (protein concentration, 1.03 mg/ml) was necessary.

Human IL-1 gives rise to biologically active degradation peptides in the same size range that were detected for thymotaxin and the 4-kDa products (23). Furthermore, IL-1 is chemotactic for mature T and B lymphocytes (24). Therefore, recombinant human IL-1 was tested for chemotactic migration of day 3 cultured bone marrow cells in Boyden chambers. None of the tested IL-1 concentrations (from 10 \( \mu g \) to 0.1 ng) attracted cultured bone marrow cells (data not shown).

Purified thymotaxin and the 4-kDa material were then tested for two known biological IL-1 activities. Neither lymphocyte activation on fresh thymocytes nor cytotoxic activity on L929-co target cells (same test used for detection of tumor necrosis factor activity) was found (Fig. 4).

**In Vitro Differentiation of Hemopoietic Precursors.** The rudiments of 14-day-old rat embryonic thymus were explanted and cultured in microwell dishes. At this stage of embryogenesis, the rat thymus contains only very few lymphocytes. In a medium specially designed for this purpose (14, 20), epithelial cells form a halo around the explants after 3 days of culture (Fig. 5a). Cultured hemopoietic precursors that responded to thymotaxin were cocultured on these thymic monolayers after a change to steroid-free medium for an additional 3-day period (Fig. 5b). Cocultured cells expressed de novo the CD8 antigen detected by Ox-8 antibodies (Fig. 5c and d; 13.5% Ox-8 positive cells). This T-cell antigen was totally absent before coculturing on embryonic thymus. Neither migrated hemopoietic cells cultured alone on tissue culture plastic in the same medium, migrated cells toward DMEM control medium, nor the thymic tissue alone produced cells bearing T-cell markers (unpublished data).

### Table 2. Cytological phenotype of migrated cells

<table>
<thead>
<tr>
<th>Cell source</th>
<th>Lymph.</th>
<th>Imm. blasts</th>
<th>Eryth. progs.</th>
<th>Myel. progs.</th>
<th>PMN</th>
<th>Monoc. macro.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before migration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>After BSA gradient</td>
<td>29 ± 1</td>
<td>12 ± 2</td>
<td>46 ± 3</td>
<td>9 ± 3</td>
<td>2 ± 1</td>
<td>0</td>
</tr>
<tr>
<td>After 3-day culture</td>
<td>41 ± 5</td>
<td>2 ± 2</td>
<td>0</td>
<td>28 ± 2</td>
<td>17 ± 4</td>
<td>12 ± 3</td>
</tr>
<tr>
<td>After migration toward</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total IT-45 R1 medium</td>
<td>51 ± 6</td>
<td>0</td>
<td>12 ± 5</td>
<td>35 ± 8</td>
<td>2 ± 1</td>
<td></td>
</tr>
<tr>
<td>Thymotaxin</td>
<td>75 ± 7</td>
<td>0</td>
<td>17 ± 8</td>
<td>9 ± 6</td>
<td>2 ± 1</td>
<td></td>
</tr>
<tr>
<td>DMEM control</td>
<td>22</td>
<td>0</td>
<td>0</td>
<td>67</td>
<td>6</td>
<td>5</td>
</tr>
</tbody>
</table>

Bone marrow-derived cells were taken before and after chemotactic migration toward IT-45 R1 conditioned medium, purified thymotaxin, and control DMEM. These cells were then cytocentrifuged and stained in May–Grünwald and Giemsa solution. Numbers represent the percentage of at least 250 counted cells per experiment and standard deviation was given for three independent experiments.

### Fig. 4. Detection of IL-1 activities in purified IT-45 R1-derived peptide fractions. (A) Mouse thymocyte activation served as detection assay for IL-1 activity. (B) Cytotoxic activity was tested by using L929 target cells. Positive controls: IL-1 activity produced by lipopolysaccharide-stimulated monocytes; *, thymotaxin; o, thymic chemotactic peptides of 4 kDa; □, negative controls: fraction that eluted at 80 ml from the G-50 column and that showed no chemotactic activity. In both assays, the thymic chemotactic molecules were negative at dilutions of optimal chemotactic activity.

### Fig. 5. Differentiation of hemopoietic precursors by coculture with embryonic thymic monolayers. Embryonic thymuses of 14- to 15-day-old rats were explanted and cultured for 3 days before coculture with hemopoietic cells was initiated. (a) A monolayer of epithelial cells grew out of the organ. (b) Coculture of migrated hemopoietic cells with embryonic thymus. (c) and (d) Phase contrast and corresponding immunofluorescence for the T-cell antigen CD8 of migrated hemopoietic precursors after differentiation by coculture on thymus.
DISCUSSION

Our results suggest that in mammals a chemotactic mechanism underlies the colonization of the thymus by hemopoietic precursors. The polypeptide thymotaxin, produced by a rat thymic epithelial cell line, attracted hemopoietic precursors present in the juvenile bone marrow in an in vitro migration assay. Responding cells of the bone marrow were enriched in a short-term coculture system by using steroid-free serum on a mouse bone marrow feeder layer. These migratory precursors had a lymphoid cytotype and acquired a T-cell marker when cocultured on monolayers of embryonic thymus.

In our assays, molecules with different size secreted by the thymic epithelium induced a chemotactic migration on hemopoietic precursors. IL-1 produces a similar molecular mass pattern of peptides as we described for the thymic epithelial cell-derived substances (23). In addition, IL-1 has a chemotactic effect on differentiated lymphoid cells (24), and it can be produced by different epithelial cells such as A431 carcinoma or human keratinocytes (25). The rat thymic peptides were tested for IL-1 activity. Both tests lead to the conclusion that IL-1 activity did not correlate with the biologically active thymotaxin. Production of thymotaxin seems to be restricted to thymic epithelial cells because other epithelial cell lines, several carcinomas, a thymic cell line with a fibroblastic phenotype, and spleen cells do not produce it (unpublished data).

The 6.2-μm hemopoietic precursors responded to the thymic peptides precisely at 3 days of culture. In prolonged cultures, these precursors disappeared and a larger cell type, expressing high peroxidase activity was generated; these larger cells responded to a variety of molecules other than those specifically secreted by the thymic epithelium (data not shown). Media selective for the emergence of different hemopoietic lineages have been developed. We have modified the xenogeneic culture system described by Hayashi et al. (19) since it was suggested that pre-T cells could be preferentially enriched under these conditions. In contrast to the steroid-rich Dexter cultures, which give rise to myeloid cells (26), steroid low culture conditions favor the maintenance or the development of lymphoid cells (19, 27). So far, culture conditions allowing the propagation of a pure population of committed pre-T cells are not yet available, although cells with different degrees of maturation in the myeloid or lymphoid lineage can be maintained (28). It is remarkable that Dexter cultures, when shifted to the Whitlock-Witte condition, provide pre-B cells after 2 weeks (28, 29). These results may reflect the survival of multipotential stem cells for lymphoid and myeloid lineages. In the present work, the rat bone marrow cells cultured on mouse stroma in steroid-free medium may lead after short-term culture to a high percentage of immature lymphoid cells capable of responding to the thymic chemotactic peptides. However, this response was limited to days of culture. This could be due to (i) an eventual limitation of responsiveness that corroborees with a defined early state of differentiation of pre-T cells and that cultured cells in many systems undergo such first events very rapidly (30), (ii) cells that only in a certain state of the cell cycle express receptors for thymic chemotactic peptides, (iii) the fact that the lymphoid cells were diluted very rapidly by fast-growing myeloid cells. Nevertheless, short-term cultures of bone marrow cells of lymphoid type facilitated our migration assay. It made the assay more reproducible because of the increased percentage of migrating cells, and it made it more independent from the condition of animal keeping; often bone marrow cells from rats stressed by transportation no longer responded; however, these animals were able to recover after 2 or 3 days.

Thymotaxin selects for small Thy-1+ lymphoid cells, which could acquire a T-cell marker in vitro. In rats, the Thy-1 molecules are expressed by precursors that give rise to a variety of hemopoietic lineages. Recent experiments in our laboratory showed that a minor population of fresh bone marrow cells enriched by chemotactic migration toward thymotaxin can also differentiate into T cells in coculture with thymic stroma. The responding cells did not contain the granulocyte, erythroid, macrophage colony-forming cells (GEM-CFC) as assayed in semisolid cultures in the presence of IL-1, IL-3, and erythropoetin (unpublished data). Therefore, thymotaxin might specifically attract committed pre-T cells; alternatively, it is intriguing to consider that it acts on stem cells, which could not reach the GEM-CFC stage in our semisolid culture system.

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