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Analysis of thymic stromal cell subpopulations grown *in vitro* on extracellular matrix in defined medium

IV. CYTOKINES SECRETED BY HUMAN THYMIC EPITHELIAL CELLS IN CULTURE AND THEIR ACTIVITIES ON MURINE THYMOCYTES AND BONE MARROW CELLS

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SUMMARY

In previous reports we described our approach to the cultivation of murine and human thymic epithelial cells in primary cultures, using defined, serum-free growth factor-supplemented medium and extracellular matrix-coated culture plates. The cells in these cultures displayed high metabolic activity and their supernatant was highly active on thymocytes. In the study reported here we analysed cytokine activities in the supernatant of human thymic epithelial cell cultures (HTES), by using the respective cytokine-dependent cell lines and by neutralization with specific monoclonal antibodies. Three cytokine activities were detected—interleukin-6 (IL-6), granulocyte colony-stimulating factor (G-CSF) and macrophage (M)-CSF. Other cytokine activities tested for [IL-1, IL-2, IL-7, interferon (IFN) and tumour necrosis factor (TNF)] were negative. The effect of HTES on concanavalin A (Con A)-induced proliferation of murine thymocytes could be completely abolished by anti-IL-6 antibodies, but not by antibodies to CSF, whereas enhancement of bone marrow cell proliferation by HTES was partially inhibited by either anti-G-CSF or anti-M-CSF antibodies and completely inhibited by both antibodies, but not at all by anti-IL-6. We can thus distinguish between thymocyte-related cytokines (IL-6) and bone marrow (myeloid/monocyte) related ones (G-CSF, M-CSF) in HTES.

INTRODUCTION

The epithelial component of the thymic stroma imparts maturation signals essential for T-cell development in two ways: direct contact with the differentiating thymocytes and secretion of a family of cytokines and other thymic soluble factors.[1–6] The maturational effects are measured *in vitro* by analysis of T-cell differentiation markers, in particular Thy-1, CD4, CD8 and CD3, and by tests for mature T-cell functions (e.g. response to mitogens by proliferation and lymphokine production). In addition, the thymic stroma might be involved in thymic myeloid cell (i.e. macrophage/monocyte) growth and maturation.[7–9] In previous work we developed a new approach for culturing primary murine and human thymic epithelial cells.[10–13] The growth of these cells was accomplished using extracellular matrix (ECM)-coated plates and serum-free growth factor-supplemented medium. The epithelial nature of the cultured cells was defined by positive staining with anti-keratin antibodies and by the presence of desmosomes and tonofilaments.

In the present study we analysed the activities of human thymic epithelial cell (HTE) culture supernatant in two complementary ways: we first defined the cytokines secreted by HTE cells and present in the culture supernatant (HTES). We then evaluated the involvement of these cytokines in HTES activities on murine thymocytes and bone marrow cells, by neutralizing the measured activities with specific anti-cytokine monoclonal antibodies.

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MATERIALS AND METHODS

Mice

C57BL/6 male mice, 4–6 weeks old were purchased from Olac Ltd (Bicester, U.K.) and kept under standard conditions.

Reagents

RPMI-1640 and Dulbecco's Modified Eagle's (DMEM) media were purchased from Gibco (Grand Island, NY). Penicillin and streptomycin were from Teva Pharmaceutics (Peta Tiqwa, Israel) and fungizone from Squibb and Sons (Princeton, NJ). Foetal calf serum (FCS), L-glutamine, sodium pyruvate and non-essential amino acids (NEAA) were from Biological Industries (Beth Haemek, Israel). 2-mercaptoethanol (2-ME) was from Fluka (Buchs, Switzerland). Concanavalin A (Con-A) was from Bio Makor (Rehovot, Israel). [^{3}H]Thymidine was from Nuclear Research Center-Negev (Beer Sheva, Israel). Tissue culture vessels were from Sterlin (Feltham, U.K.), except for the

ECM-coated culture plates which were from Eldan-Tec (Jerusalem, Israel).

Lymphokines and antibodies

Recombinant human interleukin-1α (IL-1α) and rabbit antibodies against human granulocyte colony-stimulating factor (G-CSF) were purchased from Genzyme (Boston, MA). Recombinant human IL-2 was purchased from Cetus Corporation (Emeryville, CA). Recombinant human IL-6 and mouse monoclonal antibodies (mAb) against human IL-6 were kindly provided by Interpharm (Ness Ziona, Israel) and recombinant human G-CSF by Dr S. Merchav (Technion, Haifa, Israel); recombinant human M-CSF and mAb against human M-CSF were a gift from Dr R. Apte (Ben Gurion University, Beer Sheva, Israel), rat mAb against murine IL-3 from Dr Pearce (Palo Alto, CA) and rat anti-mouse Fc receptor (FcR, 2.4G2) from J. Titus (Experimental Immunology Branch, NCI). Fluorescein isothiocyanate (FITC)–anti-Thy-1 antibody was purchased from Becton Dickinson Immunocytometry system (Mountain View, CA) and FITC–rabbit anti-rat IgG and FITC–mouse anti-bovine keratin (K8.13) were purchased from Bio Makor (Rehovot, Israel).

Primary cultures of thymic epithelial cells

Thymic tissue was obtained aseptically from children undergoing corrective cardiovascular surgery (Sheba Medical Center, Tel Hashomer and Ichilov Hospital, Tel-Aviv, Israel). HTE cell cultures were initiated by an explant technique and were grown on ECM-coated plates in defined medium-supplemented with growth factors, as previously described.[10,11,13] The cultures were 95% keratin positive, as determined by indirect immunofluorescent staining using K8.13 antibody. HTES was harvested every 2 days from confluent cultures, clarified by centrifugation and stored at −20°.

Staining for cell surface markers

FcR and Thy-1 markers stained either directly with FITC-conjugated anti-Thy-1 or indirectly with anti-FcR (2.4G2) and FITC-rabbit anti-rat antibody. Staining was done as previously described.[12,14] Briefly, samples of 2×10^6 cells were incubated with each antibody in 100 μl Ca–Mg-free phosphate-buffered saline (PBS), containing 0·1% bovine serum albumin and 0·01% sodium azide (FACS media) for 30 min at room temperature, then washed once, resuspended in 0·5 ml of FACS media, and analysed (1×10^4 cells) by a fluorescence-activated cell sorter (FACS 440; Becton-Dickinson). The parameters measured were cell size (forward light scatter) and level of surface antigen expression (fluorescence intensity). Dead cells were routinely excluded from analysis on the basis of light scatter and all the data were expressed in log arbitrary units. As control we used cells stained with the second fluorescinated antibody alone.

Cytokine assays

All assays were done in 96-well flat-bottom microtitre plates in RPMI-1640 medium containing 10% FCS [tumour necrosis factor (TNF) assay was done in 1% FCS], penicillin 100 μg/ml, streptomycin 100 μ/ml, mM glutamine, 2×10^5 M 2-ME, 0·1 mM non-essential animo acids and 1 mM sodium pyruvate. Proliferation was measured by adding [^{3}H]thymidine for the last 18 hr, harvesting and radioactivity determined by using Betamatic Kontron beta counter.[12,14] The standard deviation in all the assays described did not exceed 10%.

Details of the specific cytokine assays are as follows:

IL-2, G-CSF or *M-CSF* activities were assayed on CTLL-2, 32D or 14M1.4 (kindly provided by Dr D. Zipori (Weizmann Institute of Science, Rehovot, Israel) cell lines, respectively. 1×10^4 cells/well, were incubated for 48 hr with various dilutions of HTES, or with the relevant cytokine (rhIL-2, rhG-CSF or rhM-CSF, respectively), and proliferation measured as above. IL-2 activity was tested also be ELISA method using Biokine IL-2 test kit (Cambridge, MA).

IL-1 assay is based on the ability of CTLL-2 to respond to IL-2 secreted by EL4-NOB1 cells (purchased from the PHLS tissue culture collection, Porton, U.K.) stimulated by IL-1. 1×10^5 EL4-NOB1 cells + 5×10^3 CTLL-2 cells/well were co-cultured in the presence of various dilutions of HTES or rhIL-1α for 48 hr.

IL-6 assay. 2×10^3 B9 cells/well (kindly provided by Dr S. Rudikoff, NIH, Bethesda, MA) were incubated for 72 hr with various dilutions of HTES or rhIL-6.

IL-7 activity was measured using its ability to induce thymocyte proliferation directly without the addition of mitogen. 2×10^5 thymocytes/well were incubated with various dilutions of HTES or rhIL-7 for 72 hr.

TNF activity was measured by assaying the cytotoxicity of tested sups on the murine target cells LM (a derivative of the cell line L929, obtained from ATCC-No. CCL 23) as described previously.[15] TNF levels were defined against a known standard as the reciprocal of the dilution resulting in 50% cell cytotoxicity.

IFN activity was measured using a cytopathic effect reduction assay. This assay is based on the ability of IFN to protect HeP2 cell line from the cytophatic effect of the vesicular stomatitis virus (VSV) as previously described.[16]

Cytokine activities which were found to be positive in the respective bioassays were titrated in comparison to the recombinant cytokine and neutralized by using the respective monoclonal antibody in a dose sufficient to neutralize the titrate amount of the cytokine or more.

Effect of HTES on murine thymocytes and bone marrow (BM) cells

Co-mitogenic assay with murine thymocytes. 1×10^5 thymocytes/well were incubated with Con A (2·5 μg/ml) and with various dilutions of HTES or rhIL-6 for 72 hr. Proliferation was measured by [^{3}H]thymidine incorporation.

BM proliferation assay. BM cells (2×10^5/well) were incubated for 96 hr and [^{3}H]thymidine was added for the last 18 hr.

Staining for non-specific esterase (NSE) was done as previously described.[17]

RESULTS

Cytokine activities in HTES

Interleukins

We assayed HTES for the presence of four interleukins (IL-1, IL-2, IL-6, IL-7); only one (IL-6) was found to be present at a concentration of 2000–9000 U/ml (Fig. 1 and Table 1). IL-1 activity was assayed on EL4-NOB1 cells. The assay was sensitive up to 0·015 U/ml of rhIL-1. IL-2 was tested by two different methods: on the IL-2-dependent cell line, CTLL-2,

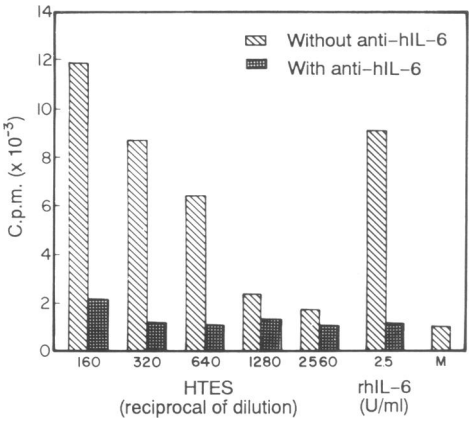

Figure 1. Effect of HTES on B9 cells. HTES or rhIL-6 were preincubated with anti-hIL-6 antibody or with control medium (M) for 1 hr before the addition of B9 cells. The proliferative response was assayed after 72 hr by pulsing with 1 μCi/well [^{3}H]thymidine for the last 4 hr of culture harvesting and counting.

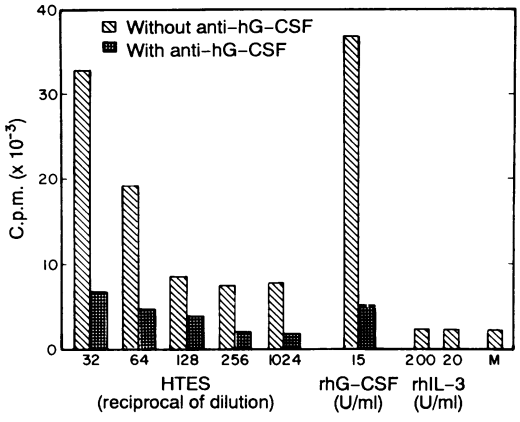

Figure 2. Effect of HTES on 32D cells. HTES or rhG-CSF were preincubated with or without anti-hG-CSF antibody for 30 min before the addition of 32D cells. The proliferative response was measured after 48 hr, by pulsing with [^{3}H]thymidine for the last 18 hr.

Table 1. Analysis of cytokines in HTES

Cytokine	Cells used for assay	Activity
IL-1	EL4-NOB1	–
IL-2	CTLL2	–
IL-6	B9	+
IL-7	Thymocytes	–
G-CSF	32D	+
M-CSF	14M1.4	+
IFN	Hep2	–
TNF	LM	–

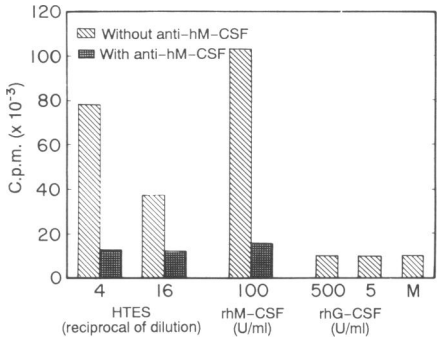

Figure 3. Effect of HTES, rhM-CSF and rhG-CSF on 14M1.4 cells and neutralization of this activity by anti-hM-CSF antibody. Conditions of the incubation and assays as in Fig. 2.

sensitive up to 0·1 U/ml of IL-2 and in a radioimmunoassay (RIA), sensitive up to 0·05 U/ml of rhIL-2. We could not detect IL-2 by either assay. Moreover, no IL-2 activity was detected even after 30-fold concentration of HTES by ultrafiltration.

The ability of IL-7 to induce thymocyte proliferation without the need of mitogens was used to evaluate the presence of this cytokine in HTES. No proliferation was seen with HTES, while rhIL-7 induced dose-dependent proliferation of the thymocytes up to 1 U/ml. On the other hand HTES had a clear, strong activity on the IL-6-dependent B-cell hybridoma line B9. This activity could be completely abrogated by anti hIL-6 antibodies (Fig. 1).

Colony-stimulating factors

HTES was found to induce proliferation of the murine BM cell line 32D in a dose-dependent fashion. This line is known to be dependent on murine IL-3, but does not respond to human IL-3. It responds, however, to human G-CSF (Fig. 2). HTES induced proliferation in 32D cells. The dose concentration of G-CSF in HTES was 100–400 U/ml. This activity could be ascribed to G-CSF, since it could be neutralized by anti-hG-CSF antibodies (Fig. 2). HTES was also found to have a stimulatory activity on the macrophage cell line 14 M1.4, which responds to M-CSF, but not to G-CSF (Fig. 3). Indeed, preincubation of HTES with anti-hM-CSF antibody completely abrogated this activity.

Other cytokines

Two other cytokine activities were looked for in HTES: TNF and IFN. Such activities could not be detected, in spite of the fact that the assays for both cytokines are highly sensitive.

HTES activity on murine thymocytes and bone marrow cells

The target cells for factors secreted by thymic stromal cells should conceivably be primarily T-lineage cells at different stages of maturation—starting with progenitor cells in the bone marrow and continuing with several stages of differentiation in the thymus. In addition, the thymus contains macrophages which originate from the bone marrow. We therefore tested HTES activity on both bone marrow cells and thymocytes.

When proliferative response (PR) was taken as an indicator for HTES activity, we found that HTES was not mitogenic by itself for thymocytes. However, it strongly augmented PR to

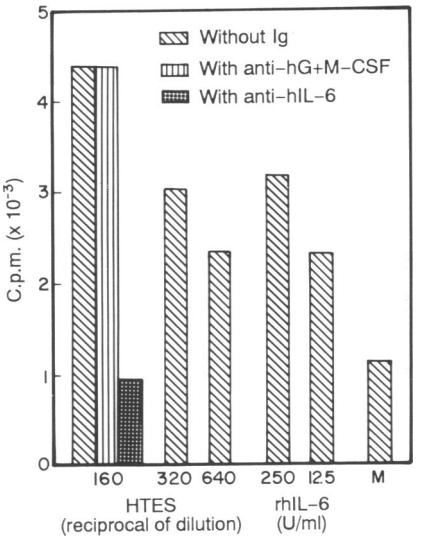

Figure 4. Titration of co-mitogenic activity of HTES and rhIL-6 on murine thymocytes; inhibition by anti-hIL-6 antibody. HTES was pretreated with anti-hIL-6, with anti-hG+M-CSF antibodies or with control medium for 1 hr before the addition of thymocytes and Con A (2·5 μg/ml). Proliferative response was assayed as described in Fig. 2.

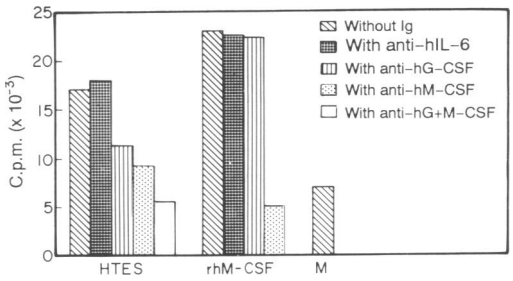

Figure 5. Effect of HTES on murine BM cell proliferation. HTES or rhM-CSF were pretreated with anti-hIL-6, hG-CSF, hM-CSF, combination of G+M-CSF antibodies, or with control medium 1 hr before the addition of BM cells. Proliferative response was measured after 96 hr as described in Fig. 2.

Con A. This co-mitogenic activity could be completely abrogated by preincubation of HTES with anti-hIL-6, but not by antibodies against G-CSF and M-CSF (Fig. 4). These data suggest that the co-mitogenic activity of HTES is related to IL-6, shown to be present in the supernatant.

The myelopoietic potential of HTES was tested by its capability to induce proliferation and differentiation of bone marrow cells. Indeed, HTES induced proliferation of mouse bone marrow cells (Fig. 5). This activity was partially neutralized by anti-hG-CSF or anti-hM-CSF antibodies and completely abrogated by both antibodies. In contrast, anti-hIL-6 antibody had no effect in this assay (Fig. 5). Four-day incubation of bone marrow cells with HTES resulted in an increase in the number of cells expressing FcR, stained for NSE, and a decrease in the number of cells expressing Thy-1. Similar results were obtained by incubating bone marrow cells with hM-CSF (Fig. 6). This co-ordinated change in FcR, NSE and Thy-1

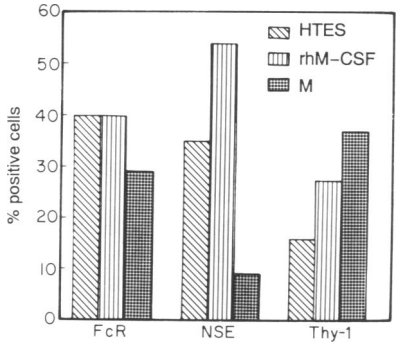

Figure 6. Effect of HTES on BM differentiation markers. BM cells were incubated with HTES, rhM-CSF or with control medium (M) for 96 hr. The cells were washed and stained for FACS analysis with anti-FcR, followed by FITC-conjugated anti-rat antibody, with FITC-conjugated anti-Thy-1 antibody, or prepared for NSE staining.

expression suggests that under HTES influence, immature Thy-1$^+$ myeloid precursor cells differentiate into more mature Thy-1$^-$ NSE and FcR$^+$ cells, of the myeloid lineage.

DISCUSSION

The main finding of the present study was the definition of known cytokine activities in HTES and the distinction between thymocyte-related cytokine (IL-6) and bone marrow (myeloid/monocyte) related ones (GM-CSF) in this supernatant. This study completes a previous one in this series,[13] which described culture conditions for HTE cells and initial observations on HTES activity.

Cytokine activities were tested on a panel of cytokine-dependent cell lines. When positive, each activity was neutralized by mAb specific to the respective cytokine. This is a critical step since most cell lines used in cytokine assays respond to more than one cytokine.

Our results demonstrate that HTES contains IL-6, G-CSF and M-CSF activities, as tested on B9, 32D and 14M1.4 cell lines, respectively. These activities were completely neutralized by the respective mAb. On the other hand, we did not find activities of IL-1, IL-2, IL-7, IFN and TNF in sensitive assays. Indeed, Le *et al.* reported that human thymic epithelial cells contained mRNA transcripts for IL-6, G-CSF and M-CSF and their supernatant showed the corresponding activities.[7,8] However, their assay for G-CSF and M-CSF was based on the identification of BM colonies, an assay which is not entirely specific. For example, G-CSF alone can induce the formation of both granulocyte and macrophage colonies. Moreover, the presence of cytokine messenger RNA (mRNA) does not mean necessarily that a secretory product is produced. Neutralization of activities by specific antibodies is needed as additional evidence.

The absence of IL-1 activity in HTES is in contrast to previous reports that demonstrate the existence of this factor in both human and mouse thymic epithelial cell supernatant.[18,19] The reported amount of IL-1 produced by the human cells is at least 2000 times higher than the sensitivity limit of our IL-1 detection assay. It is therefore unlikely that lack of sensitivity is the cause of our inability to detect IL-1 activity in HTES. The contradiction may stem from the differences in culture conditions, which may encourage the growth of different thymic

stromall cell subpopulations, only some of which produce IL-1 or stimulate epithelial cells to do so. Another possibility is that IL-1 may be bound to ECM components present in our culture plates or to the epithelial cell membranes in our cultures. ECM components were reported to bind soluble factors such as GM-CSF.[20] Hence, it may be that IL-1 is produced in our culture but cannot be detected in the supernatant.

We expected that IL-7 would be present in HTES, in view of recent reports which demonstrate the presence of IL-7 mRNA in the thymus and in some thymic epithelial cell lines[21,22] and its effects on thymocytes.[22-24] However, IL-7 mRNA has been detected only in the cortical but not in the medullary epithelial cell lines.[22] This selective transcription can explain the lack of IL-7 activity in our HTES, since most probably medullary epithelial cells are growing in our cultures.[13]

Several investigators reported that IL-3-like activity is present in human epithelial cell supernatant.[25] These reports were based on the use of murine IL-3-dependent cell lines (mostly 32D cells). However, these cell lines cannot respond to human IL-3 and, therefore, we looked for other cytokines. We found that hG-CSF is the factor responsible for the reported IL-3-like activity in our HTES.

Although GM-CSF activity was not assayed, our previous findings in the murine system[12] suggest that this CSF is also secreted by thymic epithelial cells. Indeed, other investigators reported that GM-CSF is produced by human thymic epithelial cells.

After defining the cytokines present in HTES we tested its influence on murine thymocytes and on BM cells, using neutralizing antibodies against the identified cytokines, in an attempt to ascribe HTES activities to any of these cytokines.

We demonstrated that HTES has a co-stimulatory activity on thymocytes activated by Con A. Previous reports showed that IL-6 has a co-stimulatory effect on thymocytes and mature T cells.[26,27] These results suggest that IL-6 present in HTES mediated this activity. Indeed, only anti-IL-6 antibody, but not anti G-CSF or M-CSF antibodies neutralized HTES co-stimulatory activity on thymocytes.

Although there are also some reports about the effect of G-CSF and M-CSF on T cells,[28] these cytokines are considered mainly as factors affecting myeloid cell proliferation and differentiation. Indeed, our results suggest that CSF have no direct effect on thymocytes. Obviously, CSF can act indirectly by inducing the proliferation and differentiation of thymic macrophages, which may, in turn, function as antigen-presenting cells or as cytokine-producing cells.

Bone marrow cells were used as a model for HTES effect on myeloid cells, some of which are also present in the thymus. HTES induced murine bone marrow cell proliferation. This activity could be completely neutralized by adding both anti-hG-CSF and anti hM-CSF antibodies and partially neutralized by each one. Anti-hIL-6 antibody had no effect. These results suggest that both CSF, but not IL-6, are involved in regulating myeloid cell proliferation in our system. Although IL-6 alone or with CSF had been reported to act on myeloid cells,[29,30] in our assay rhIL-6 had a minor influence on bone marrow cell proliferation and anti-IL-6 antibody failed to neutralize HTES effect on these cells. Differences in experimental conditions or in test parameters (e.g. cell proliferation in our work and bone marrow colony formation in other studies) may explain this contradiction. Long-term incubation of bone marrow cells with HTES resulted in an increase in the percentage of cells expressing mature myeloid cell markers, FcR and NSE activity and a decrease in the percentage of cells expressing immature myeloid cell marker, Thy-1. These results indicate that HTES could regulate myeloid cell proliferation and/or differentiation.

Our present findings suggest that several cytokines, including IL-6, G-CSF and M-CSF, are secreted by human thymic epithelial cells in culture. These cytokines were found to have a pronounced influence on thymocytes as well on bone marrow cells. While IL-6 seemed to be the major factor regulating thymocyte activation by Con A, both G-CSF and M-CSF were found to be important in regulating the proliferation and differentiation of the myeloid cells.

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REFERENCES

1. Stutman Q. (1978) Intrathymic and extrathymic T-cell maturation. *Immunol. Rev.* **42,** 138.
2. Haynes B.F. (1984) The human thymic microenvironment. *Adv. Immunol.* **36,** 87.
3. Shoham J. (1984) Thymic hormones—immunological effects and implications to clinical use. In: *Recent Advances in Pediatric Immunology, Hematology and Oncology* (eds L. Massimo and P. Cornaglia Ferraris), p. 161, Piccin Medical Books, Padova, Italy.
4. Shoham J. (1986) The role of thymic hormones in regulating T cell activity and lymphokine production. In: *Recent Advances in Primary and Acquired Immunodeficiencies* (eds F. Aiuti, F. Rosen and M. D. Cooper), p. 101. Raven Press, New York.
5. Schulof R.S., Naylor P.H., Sztein M.B. & Goldstein A.L. (1987) Thymic physiology and biochemistry. *Adv. clin. Chem.* **26,** 203.
6. Boyd R.L. & Hugo P. (1991) Towards an integrated view of thymopoiesis. *Immunol. Today*, **12,** 71.
7. Le P.T., Lazorick S. Whichard L.P., Yang Y-C., Clark S.C., Haynes B.F. & Singer K.H. (1990) Human thymic epithelial cells produce IL-6, granulocyte-monocyte-CSF, and leukemia inhibitory factor. *J. Immunol.* **145,** 3310.
8. Le P.T., Kurtzberg J., Brandt S.J., Niedel J.E., Haynes B.F. & Singer K.H. (1988) Human thymic epithelial cells produce granulocyte and macrophage colony-stimulating factors. *J. Immunol.* **141,** 1211.
9. Izon D.J., Boyd R.L. Waanders G.A. & Kelso A. (1989) The myelopoietic inducing potential of mouse thymic stromal cells. *Cell Immunol.* **124,** 264.
10. Shoham J. (1991) Characterization of murine thymic nonlymphoid cell subpopulations and their secretory products *in vitro*. In: *Lymphatic Tissues and* in vivo *Immune Responses* (eds B. A. Imhof, S. Berrik-Aknib and A. S. Ezine), p. 75. Marcel Dekker, New York.
11. Eshel I., Savion N. & Shoham J. (1990) Analysis of thymic stromal cell subpopulations grown *in vitro* on extracellular matrix in defined medium. I. Growth conditions and morphology of murine thymic epithelial and mesenchymal cells. *J. Immunol.* **144,** 1554.
12. Eshel I., Savion N. & Shoham J. (1990) Analysis of thymic stromal cell subpopulations grown *in vitro* on extracellular matrix in defined medium. II. Cytokine activities in murine thymic epithelial and mesenchymal cell culture supernatants. *J. Immunol.* **144,** 1563.
13. Schrieber L., Eshel I., Meilin A., Sharabi Y. & Shoham J. (1991) Analysis of thymic stromal cell subpopulations grown *in vitro* on extracellular matrix in defined medium. III Growth conditions of

human thymic epithelial cells and immunomodulatory activities in their culture supernatant. *Immunology*, **74,** 621.

14. Savion S., Itoh T., Hertoges H. & Shoham J. (1989) Contract-mediated maturational effects of thymic stromal cells on murine thymocytes in culture. *Immunology*, **67,** 496.
15. Flick D.A. & Gifford G.E. (1984) Comparison of *in vitro* cell cytotoxic assays for tumor necrosis factor. *J. immunol. Meth.* **68,** 167.
16. Havell E.A. & Vilcek J. (1972) Production of high-titered interferon in cultures of human diploid cells. *J. Antimicrob. Agents Chemother.* **2,** 476.
17. Tucker S.B., Pierre R.V. & Jordon R.E. (1977) Rapid identification of monocytes in a mixed mononuclear cell preparation. *J. immunol. Meth.* **14,** 267.
18. Le P.T., Tuck D.T., Dinarello C.A., Haynes B.F. & Singer K.H. (1987) Human thymic epithelial cells produce interleukin 1. *J. Immunol.* **138,** 2520.
19. Farr A.G., Hosier S., Braddy S.C., Anderson S.K., Eisenhardt D.J., Jie Yan Z. & Robles C.P. (1989) Medullary epithelial cell lines from murine thymus constitutively secrete IL-1 and hematopoietic growth factors and express class II antigens in response to recombinant interferon-γ. *Cell. Immunol.* **119,** 427.
20. Roberts R., Gallagher J., Spooncer E., Allen T.D., Bloomfield F. & Dexter T.M. (1988) Heparan sulphate bound growth factors: a mechanism for stromal cell mediated haemopoieses. *Nature*, **332,** 376.
21. Namen A.E., Lupton S., Hjerrild K., Wignall J., Mochizuki D.Y., Schmierer A.E. *et al.* (1988) Stimulation of B cell progenitors by cloned murine interleukin 7. *Nature*, **333,** 571.
22. Gutierrez J.C. & Palacios R. (1991) Heterogeneity of thymic epithelial cells in promoting T-lymphocyte differentiation *in vivo*. *Proc. natl. Acad. Sci. U.S.A.* **88,** 642.
23. Watson J.D., Morrissey P.J., Namen A.E., Conlon P.J. & Widmer M.B. (1989) Effect of IL-7 on the growth of fetal thymocytes in culture. *J. Immunol.* **143,** 1215.
24. Groh V., Fabbi M. & Strominger J.L. (1990) Maturation or differentiation of human thymocyte precursors *in vitro? Proc. natl. Acad. Sci. U.S.A.* **87,** 5973.
25. Tanno Y., Stadler B. & Denburg J.A. (1987) Human interleukin-3 like activity, basophil and eosinophil growth promoting activities and colony stimulating factor derived from several cell lines. *Int. Archs Allergy appl. Immunol.* **83,** 1.
26. Lotz M., Jirik F., Kabouridis P., Tsoukas C., Hirano T., Kishimoto T. & Carson D.A. (1988) B cell stimulating factor 2/interleukin 6 is a costimulant for human thymocytes and T lymphocytes. *J. exp. Med.* **167,** 1253.
27. Uyttenhove C., Coulie P.G. & Van Snick J. (1988) T cell growth and differentiation induced by interleukin-HP1/IL-6, the murine hybridoma/plasmacytoma growth factor. *J. exp. Med.* **167,** 1417.
28. Valteiri M., Santoli D., Caracciolo D., Kreider B.L., Altmann S.W., Tweardy D.J., Gemperlein I., Mavilio F., Lange B.J. & Rovera G. (1987) Establishment and characterization of an undifferentiated human T leukaemia cell line which requires granulocyte–macrophage colony stimulating factor for growth. *J. Immunol.* **138,** 4042.
29. Ikebuci K., Wong G.G., Clark S.C., Ihle J.N., Hirai Y. & Ogawa M. (1987) Interleukin-6 enhancement of interleukin-3-dependent proliferation of multipotential hemopoietic progenitors. *Proc. natl. Acad. Sci. U.S.A.* **84,** 9035.
30. Wong G.G., Witek-Giannotti J.S., Temple P.A., Kriz R., Ferenz C., Hewick R.M., Clark S.C., Ikebuchi K. & Ogawa M. (1988) Stimulation of murine hemopoietic colony formation by human IL-6. *J. Immunol.* **140,** 3040.