An Extract of Bovine Thymus Stimulates Human Keratinocyte Growth In Vitro

Betzabé M. Stanulis-Praeger, Ph.D., Mina Yaar, M.D., Gerard Redziniak, Ph.D., Alain Meybeck, Ph.D., and Barbara A. Gilchrest, M.D.

United States Department of Agriculture Human Nutrition Research Center on Aging at Tufts University, Boston, MA, and Moet-Hennessy Recherche, Colombes, France.

An extract prepared from newborn calf thymus stimulated proliferation of human keratinocytes cultured from newborn foreskins and from skin biopsies of 26 adult volunteers aged 19 to 70 years. Growth over the 7-day assay period in the basal medium was age-dependent, with newborn cultures achieving a 10-fold increase in cell number over seeding density, old adult cultures barely maintaining their seeding density and young adult cultures intermediate in proliferative capacity. Maximally stimulatory extract concentration was 5-fold higher for newborn than for adult keratinocytes, with adult cultures experiencing toxicity at doses still growth-promoting for newborn cultures. At optimal extract concentration the maximal average increase in cell yield (66.3% for newborn, 53.6% for young adult, and 18.1% for old) indicated decreased mitogen responsiveness or increased inhibitor sensitivity with increasing donor age. Stimulation of cholera toxin-treated cultures was equally high, ranging from 39.4% to 145.9%, suggesting that the extract acts through a cyclic AMP-independent pathway. Thymic extract did not increase colony forming efficiency. Our findings provide further support for the concept of functional interactions between the skin and the immune system, in addition to the recognized morphologic similarities between thymic cells and keratinocytes. Furthermore, these data confirm earlier findings of an inverse relationship between mitogen responsiveness and donor age for cultured cells. *(J Invest Dermatol 90: 749–754, 1988)*

Recent work by several groups has established a close and interactive relationship between the skin, particularly the epidermis, and the immune system. It is now known that Langerhans cells, constituting approximately 3% of the epidermal cell population, are bone marrow-derived antigen presenting cells required for topical allergic sensitization and probably for cutaneous immune surveillance generally [1–3]. Certain thymus-derived (T) lymphocyte subsets have a strong orientation toward the skin [4,5] and indeed may require an epidermal environment to complete their maturation [6,7]. Malignancies of such T cells are manifested initially and principally in the skin [4], losing their epidermotropism only late in the de-differentiation process. A separate line of investigation has shown that epidermal keratinocytes produce thymopoietin [8], a factor originally isolated from the thymus and required for T-lymphocyte differentiation. Keratinocytes are also known to produce several cytokines in the interleukin family [9–13], particularly epidermal cell-derived thymocyte-activating factor (ETAF) [11,12] that is highly similar or identical to interleukin-1 [11,13,14], also recently demonstrated in cultured keratinocytes using northern blot analysis [13,15].

Based on these observations and the long-suspected importance of the thymus gland to the aging process [16–18], the following studies were undertaken to determine whether the thymus gland might exert an influence on cultured human keratinocytes and whether this influence might depend on the age of the keratinocyte donor.

MATERIALS AND METHODS

Cell Source Twenty-six healthy adult volunteers aged 19 to 70 years provided 6-mm-diameter cutaneous punch biopsies from the medial aspect of the upper arm, and newborn foreskins were obtained at the time of elective circumcision. All tissue specimens were processed according to a modification of the Rheinwald-Green method [19] to yield primary keratinocyte cultures. Briefly, foreskins were mechanically separated into dermal and epidermal fragments after incubation overnight in 0.25% trypsin at 4°C. Epidermal fragments were then incubated in 0.2% EDTA, vortexed, and centrifuged at 240 × *g* for 5 minutes. The pellet was resuspended in Dulbecco's Modified Essential Medium (DMEM) containing 20% fetal bovine serum (FBS) and plated in 60-mm dishes containing a lethally irradiated 3T3 fibroblast feeder layer and 4-ml DMEM containing 20% FBS and 1.4×10^{-6} M hydrocortisone. Cultures were maintained at 37°C in 8% CO_2/air and provided with fresh medium twice weekly. At near-confluence, primary cultures were sprayed with 0.02% EDTA-0.25% trypsin lacking Ca^{++} and Mg^{++} to remove any remaining 3T3 cells, incubated in fresh EDTA-trypsin for approximately 10 minutes to release the keratinocytes, vigor-

Manuscript received June 22, 1987; accepted for publication November 5, 1987.

This work was supported by the Agricultural Research Service and a grant from Moet-Hennessy Recherche.

Reprint requests to: Barbara A. Gilchrest, M.D., USDA Human Nutrition Research Center at Tufts University, 711 Washington Street, Boston, MA 02111.

Abbreviations:
- CFE: colony-forming efficiency
- dH_2O: distilled water
- DMEM: Dulbecco's Modified Essential Medium
- EGF: epidermal growth factor
- ETAF: epidermal cell-derived thymocyte-activating factor
- FBS: fetal bovine serum
- IL: interleukin
- T: thymus-derived

ously pipetted to yield a single-cell suspension, treated with DMEM containing 10% FBS to inactivate the trypsin, centrifuged as previously to produce a pellet, resuspended in serum-free medium, and counted in a hemacytometer chamber.

Thymic Extract Preparation Thymus glands from one-month-old calves were minced in distilled water (dH_2O) and subjected to partial hydrolysis for 3 hours using 6 g/liter of chymotrypsin (20 units/g)-trypsin (770 units/g) (Sigma) at 37°C, pH 8. The resulting extract was then heated to 80°C for 15 minutes to stop the hydrolysis, dialyzed against dH_2O using a membrane with a pore size of 10,000 exclusion, lyophylized, stored at 4°C, and reconstituted in dH_2O or Medium 199 as a 5% (w/v) stock solution prior to use in the bioassay.

Culture Medium For all experiments keratinocytes were maintained in Medium 199 (GIBCO, Grand Island, NY) supplemented with 10 ng/ml epidermal growth factor (EGF; Bethesda Research Laboratories, Rockville, MD), 10 μg/ml insulin (Sigma, St. Louis, MO), 10^{-9} M tri-iodothyronine (Sigma), 10 μg/ml transferrin (Sigma), 1.4×10^{-6} M hydrocortisone (Calbiochem, San Diego, CA), 2 mg/ml bovine serum albumin (Sigma), and 150 μg/ml of a bovine hypothalamic extract known to contain keratinocyte growth-promoting activity [20,21]. In some experiments, 1.2×10^{-9} M cholera toxin (Calbiochem) was also added to the medium prior to addition of the thymic extract, as described below.

Experimental Design

Growth Assays In each experiment, cells were seeded at an equal density of 0.8 to 3.1×10^4/cm² in triplicate into 35-mm dishes coated with 10 μg/cm² human fibronectin [21]. Each dish contained 2 ml of the serum-free medium, supplemented with the thymic extract at a final concentration of 0.01%, 0.25%, 0.125%, 0.25%, or 0.5% (v/v). Control cultures were supplemented with an equal volume of diluent lacking thymic extract. In later experiments, the 0.125%, 0.25%, and 0.5% groups were omitted from the adult donor assays in order to conserve cells, since these higher concentrations were consistently less stimulatory than 0.025% or even toxic for the first six adult donors tested. All experimental and control cultures were maintained at 37°C in 8% CO_2 and provided with fresh medium 3 times weekly. Ten days after seeding, one dish was stained with 1% Rhondanile Blue to assess colony size [19], and two dishes were trypsinized and the cells counted with a hemacytometer. Percent stimulation was calculated as $100 \times$ [(maximal count) − (control count)]/(control count).

Colony-Forming Efficiency Skin specimens were trypsinized overnight at 4°C and then plated on a loosely confluent lethally irradiated 3T3 cell layer in 35-mm dishes at 1.1 to 3.8×10^3 cells/cm² in serum-free medium containing thymic extract (see above) at final concentrations of 0%, 0.01%, 0.25%, 0.05%, 0.125%, or 0.5%. Dishes were provided twice weekly with fresh medium. When average colony size reached approximately 1,000 cells, 3T3 cells were removed by vigorous pipetting with 0.02% EDTA for 15 seconds. The keratinocyte colonies were then fixed and stained with Rhodanile Blue and the number of colonies was determined by visual assessment.

Statistical Analysis Mean differences in percent maximal stimulation among newborn, young adult and old adult donors (Fig 1 and Table I) were tested for significance by a 2-way non-parametric one-way analysis of variance by ranks (the Kruskal-Wallis test), followed by the Wilcoxin-Mann-Whitney test for intergroup comparisons. Mean differences in cell growth between untreated and treated cells at the most stimulatory concentration of extract were tested for significance by an analysis of variance and a paired t test (Tables I and II).

RESULTS

Growth Assays Growth in the control cultures was strongly age-dependent. Newborn cultures increased in cell number approximately 10-fold over the seed inoculum and often attained confluence within the 10-day assay. In comparison, old adult cultures increased in number inconsistently and remained sparse, and young adult cultures were intermediate in growth response (Figs 1 and 2). At optimal concentrations, thymic extract stimulated the proliferation of keratinocytes from most donors in all age groups (Fig 1), although stimulation of old adult cells was not statistically significant. The optimal concentration for newborn-derived cell lines was 0.125% (w/v), whereas the average optimal concentration for adult-derived cell lines was 5-fold less or 0.025% (w/v) (Fig 1). The maximal average increase (Table I) in cell yield for each group (66.3% for newborn, 53.6% for young adult, and 18.1% for old) indicated decreased responsiveness to the thymic extract with increasing donor age, particularly between early and late adulthood ($p = 0.012$).

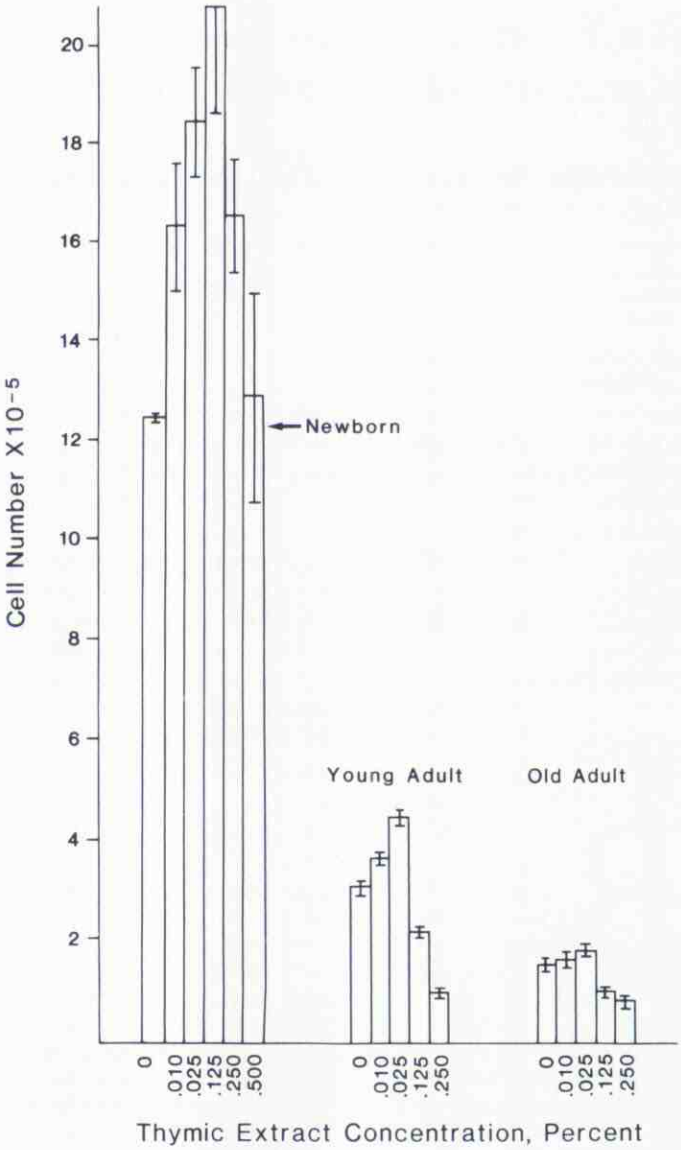

Figure 1. Effect of thymic extract at varying concentrations (% w/v) on growth of human keratinocytes from donors of varying age. *Column heights* and *bars* represent mean cell yield ± SEM 10 days after seeding for 3 to 8 donors per age group. The 0.5% concentration was toxic for the adult donor cultures and prevented meaningful cell counts. At optimal concentration, the extract provided statistically significant stimulation of newborn ($p = 0.009$) and young adult cells ($p = 0.006$), but not of old adult cells ($p = 0.374$).

In the case of the newborn donors, greater percentage of stimulation was observed in assays in which growth was relatively modest than in assays in which control dishes approached confluence. In separate experiments designed to address this phenomenon, kera-

Table I. Thymic Extract Stimulation of Human Keratinocyte Growth

Age (Years)	Cell Counts × 10^{-5} Extract % (w/v) 0	0.1	0.025	0.125[a]	%Maximal Stimulation
Newborn	12.5	14.8	20.5	25.1	100.8
Newborn	12.4	15.8	16.9	17.4	40.3
Newborn	12.8	18.8	18.4	20.2	57.8
Mean ± SEM	12.6 ± 0.12	16.5 ± 1.20	18.6 ± 1.04	20.9 ± 2.25[b]	66.3 ± 17.9
19	1.5	1.5	2.6		70.6
22	1.3	0.9	1.8		44.4
21	1.5	2.2	1.7		48.0
19	5.3	5.3	7.1		34.6
22	2.1	2.0	3.1		48.3
20	7.3	8.3	9.5		29.5
23	4.1	5.8	7.3		86.7
29	2.4	4.0	3.2		66.7
Mean ± SEM	3.2 ± 0.78	3.8 ± 0.91	4.6 ± 1.00[c]		53.6 ± 6.87
60	1.6	1.8	2.0		25.0
70	0.9	0.5	0.7		0
70	2.3	2.6	3.0		29.3
Mean ± SEM	1.6 ± 0.41	1.6 ± 0.62	1.9 ± 0.67[d]		18.1 ± 9.14[e]

[a] The 0.125% concentration was not consistently tested for the adult donors, as it was uniformly less stimulatory than the 0.025% concentration in early experiments.
[b] Counts in stimulated cultures greater than in controls ($p = 0.009$).
[c] Counts in stimulated cultures greater than in controls ($p = 0.006$).
[d] Counts in treated cultures not significantly different from controls ($p = 0.374$).
[e] Percent maximal stimulation less than for young ($p = 0.012$).

Table II. Thymic Extract Stimulation of Cholera Toxin-Treated Human Keratinocytes

Donor	Cell counts × 10^{-5} Extract (w/v) 0%	0.01%	0.025%	% Maximal Stimulation
1	0.60	0.95	1.47	143.3
2	2.73	4.16	5.35	95.9
3	2.33	3.30	3.99	71.2
4	3.89	4.54	6.81	75.1
5	11.38	17.20	21.78	91.4
6	5.19	7.31	8.63	66.3
7	1.78	3.81	4.36	145.9
8	4.19	6.78	7.09	69.2
9	6.63	9.24	6.90	39.4
Mean ± SEM	4.30 ± 1.07	6.37 ± 1.58	7.38 ± 1.93[a]	88.6 ± 11.8

[a] Counts in stimulated cultures greater than in controls ($p = 0.013$).

tinocytes from a single newborn donor were plated in triplicate at each extract concentration at two relatively low seed densities that resulted in minimal growth of control cultures. Under these conditions, the same extract concentrations stimulated keratinocyte growth up to 20-fold above controls (data not shown).

Cholera Toxin Effect In a separate study utilizing 9 adult donors, keratinocytes from cholera-toxin-exposed primary cultures responded to thymic extract in the same way as non-cholera toxin-exposed keratinocytes (Table II). Stimulation in the presence of 0.025% thymic extract was highly significant ($p = 0.013$), and the

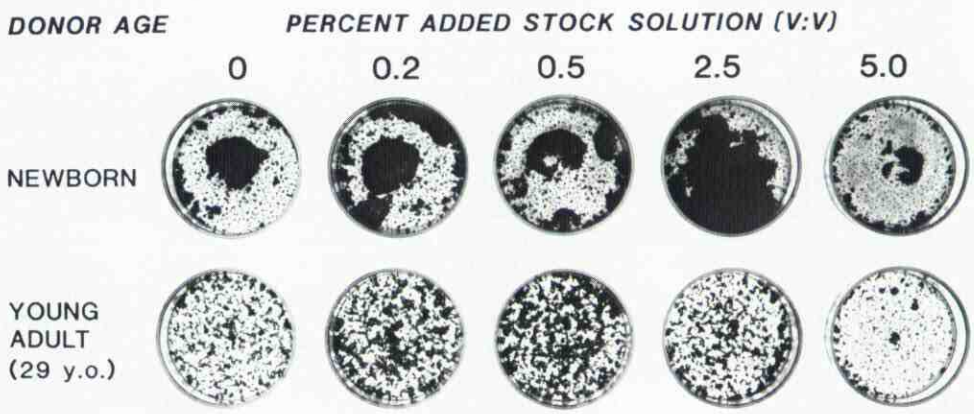

Figure 2. Response of representative human keratinocyte cultures from newborn and young adult donors to thymic extract. Percents extract stock solution (v/v) correspond to the following final percent concentrations of extract (w/v): 0.2 = 0.01, 0.5 = 0.025, 2.5 = 0.125 and 5.0 = 0.25.

Table III. Colony-Forming Efficiency of Primary Cultures

	% Thymic Extract (w/v)					
Age (Years)	0	0.01	0.025	0.05	0.125	0.50
Newborn	0.80			0.44	0.41	0.32
Newborn	0.26	0.26	0.22		0.29	
Newborn	0.13	0.13	0.13		0.06	
22	0.02	0.01	0.02		0.003	
24	0.13	0.14	0.14		0.03	
28	0.41	0.42	0.32		0.14	
60	0.59			0.21	0.08	0.02
66	0.12	0.12	0.09		0.03	

NOTE: Entries are the number of colonies at 3 weeks/number of cells seeded × 100. That is, CFE = 0.1 represents 1 colony per 1,000 cells seeded. Values are the mean of duplicate counts. Absent entries indicate no testing at that extract concentration for that donor.

percent stimulation relative to control was slightly greater than that observed in the absence of this cAMP modulator.

Colony-Forming Efficiency (CFE) Thymic extract did not stimulate CFE and indeed at higher concentration appeared to inhibit colony formation (Table III). The basal CFE of newborn, young adult, and old adult keratinocytes in primary cultures varied from 0.02% to 0.8% without a detectable age effect for the 9 donors tested. At thymic extract concentrations below 0.5%, there was also no effect on keratinocyte morphology as judged by phase-contrast microscopy.

DISCUSSION

The present study demonstrates that an extract of bovine thymus stimulates proliferation of human keratinocytes in vitro. Stimulation was more pronounced in cultures derived from newborns and young adults than from older adults and, at optimal thymic extract concentrations, was moderately higher in each age group than the 0% to 40% stimulation previously observed with epidermal growth factor in the same culture system [22–24]. The effect appeared not to be attributable to increased CFE of plated cells, at least in the feeder layer-supported primary cultures in which this parameter was assessed; and the keratinocyte attachment rate in the growth assay culture system has previously been shown to be relatively constant and independent of such factors as mitogen content of this medium or cell donor age [22,23]. Thymic extract stimulation could not be blocked by prior addition of cholera toxin, a potent long-acting enhancer of adenyl cyclase activity [25], known to increase keratinocyte yield in vitro through elevation of intracellular cAMP levels [26].

The epidermis has been found to have both morphologic and functional parallels to the immune system and to participate actively in certain immunologic reactions. Histologically, keratin proteins and keratohyalin granules, characteristic epidermal markers, have also been identified in thymic epithelial cells [7]. Functionally, cultured keratinocytes induce terminal deoxynucleotidyl transferase synthesis in immature proliferating T cells [6,7], a T-cell maturation event that otherwise occurs predominantly in the thymus. Functional analogy between the epidermis and thymus is further supported by the finding that antibody to thymopoietin, a thymic hormone produced by thymic epithelium and involved in T-cell maturation [27,28], binds to keratinocytes in the basal layer of human epidermis [8]. In addition, keratinocytes have been found to secrete ETAF, a molecule highly homologous or identical to interleukin (IL)-1 [11-15], a cytokine in turn known to be secreted by antigen-presenting cells [5,29,30]. IL-1 stimulates T cells to release IL-2, an inducer of T-cell proliferation and specificity [9,10]. The impact of keratinocytes upon T-cells is thus broad and ranges from an influence upon maturation to an enhancement of antigenic responsiveness. In light of the above, it is interesting but perhaps not surprising to find that the thymus, known to produce several thymocyte mitogens [27], also stimulates the proliferation of keratinocytes.

The thymus gland is a complex tissue containing fibroblasts, macrophages, and T lymphocytes at various stages of maturation in addition to epithelial cells. The identity and cellular origin of the factor or factors in the extract responsible for the keratinocyte growth-stimulating activity are not yet known. A partially purified heat-stable, acidic thymic extract (thymosin fraction 5) and a number of other preparations with thymic hormone-like activity have been isolated from thymus and thymic epithelial supernatants [17,27,31]. Most of the preparations are mixtures with multiple components. However, several polypeptides with T-cell activities have been isolated and characterized. These include thymosin $\alpha 1$ [32], thymosin $\beta 4$ [33], and thymopoietin [28], ranging in size from 28 to 49 amino acids; and thymulin, a zinc-containing nonapeptide [34]. Human thymic epithelial cells have also recently been found to secrete IL-1 [35], a known keratinocyte mitogen [36,37]. Although IL-1 is digested by chymotrypsin [38], the enzyme used in the preparation of the extract for the present experiments, IL-1 fragments at least 127 amino acids in length have been found to retain biologic activity [39]. That such fragments might persist after hydrolysis and remain within a dialysis tubing with a molecular weight cut-off of 10,000 daltons is unlikely but cannot be excluded. Other recently reported thymic peptides bear a high degree of functional homology to either thymosin $\alpha 1$ or $\beta 4$ [27].

Thymosin fraction 5 and thymosin $\alpha 1$ specifically stimulate T-cell proliferation by stimulating IL-2 production, and all the thymic hormones regulate differentiation of thymus-dependent lymphocytes [27,40-42]. To date only thymopoietin has demonstrated relevance to the skin, in that basal epidermal cells bind antisera to thymopoietin [8]. It is yet not known if the mitogenic activity for keratinocytes present in calf thymus extract derives from any of these known hormone-like factors nor indeed if it is epithelial in origin. Similarly, the present studies do not establish that the mitogenic activity is unique to thymus, whatever its cellular origin and biochemical character.

Some but not all defined thymocyte mitogens are believed to act through cyclic nucleotides [43,44], and addition of cyclic AMP mimics the action of thymosin in several assays [45]. Increased cyclic AMP is also known to stimulate keratinocyte growth rate and culture lifespan [26,46,47]. In the present experiments, the thymic extract stimulated keratinocyte proliferation equally effectively before and after exposure to cholera toxin. Because cholera toxin is known to increase adenyl cyclase activity and hence intracellular cyclic AMP levels [25], this suggests but does not establish that the thymic effect is mediated by a cyclic AMP-independent pathway.

Decreased mitogen responsiveness has previously been noted to accompany cellular aging, as a function of both increasing passage level for fetal lung fibroblasts [48,49] and increasing donor age for skin-derived keratinocytes and fibroblasts [24,50,51]. In the latter case, dramatic differences are generally apparent between the responsiveness of newborn and adult cultures, whereas differences between young adult and old adult cultures are more subtle [51]. The comparable degrees of stimulation noted for newborn and young adult cultures in response to thymic extract in the present

experiments may well be attributable to the excellent growth and hence near confluence of the unstimulated newborn controls in many assays, minimizing further growth even in the presence of an effective mitogen. Alternatively, the thymus-derived mitogen(s) here examined may respond to the cellular aging program in a manner distinct from that of epidermal growth factor, insulin, and the other factors examined to date.

Whether the thymus gland has a regulatory role in keratinocyte proliferation and epidermal immune competence in vivo is unknown. The availability of purified thymic growth factors, cytokines, and specific antibodies and genetic probes directed against such activities, as well as appropriate bioassay systems, should permit further exploration of this important question.

REFERENCES

1. Katz SI, Tamaki K, Sacks DH: Epidermal Langerhans cells are derived from cells which originate in bone marrow. Nature 282:324–326, 1979
2. Shelley WB, Juhlin L: Selective uptake of contact allergens by the Langerhans cell. Arch Dermatol 113:187–192, 1977
3. Stengl G, Katz SI, Clement L, Green I, Shevach EM: Immunologic functions of Ia-bearing epidermal Langerhans cells. J Immunol 121:2005-20013, 1978
4. Lutzner M, Edelson R, Schein P, Green I, Kirkpatrick C, Ahmed A: Cutaneous T-cell lymphomas: the Sezary syndrome, mycosis fungoides, and related disorders. Ann Intern Med 83:534–552, 1975
5. Streilein JW: Skin-associated lymphoid tissues (SALT): origins and functions. J Invest Dermatol 80:012S, 1983
6. Rubenfeld MR, Silverstone AE, Knowles DM, Halper JP, Sostoa A, Fenoglio CM, Edelson RL: Induction of lympocyte differentiation by epidermal cultures. J Invest Dermatol 77:221–224, 1981
7. Patterson JAK, Edelson RL: Interaction of T-cells with the epidermis. Br J Dermatol 107:117–122, 1982
8. Chu AC, Patterson JAK, Goldstein G, Berger CL, Takezaki S, Edelson, RL: Thymopoietin-like substance in human skin. J Invest Dermatol 81:194–197, 1983
9. Kupper TS, Coleman DL, McGuire J, Goldminz D: Keratinocyte-derived T-cell growth factor: a T-cell growth factor functionally distinct from interleukin 2. Proc Natl Acad Sci USA 83:4451–4455, 1986
10. Larrson EL, Iscove NN, Coutinho A: Two distinct factors are required for induction of T-cell growth. Nature 283:664–666, 1980
11. Luger TA, Stadler BM, Katz SI, Oppenheim JJ: Epidermal cell (keratinocyte)-derived thymocyte activating factor (ETAF). J Immunol 127:1493–1498, 1981
12. Sauder DN, Carter CS, Katz SI, Oppenheim JJ: Epidermal cell production of thymocyte activating factor (ETAF). J Invest Dermatol 79:34–39, 1982
13. Kupper TS, Ballard DW, Chua AO, McGuire JS, Flood PM, Horowitz MC, Langdon R, Lightfoot L, Gubler U: Human keratinocytes contain mRNA indistinguishable from monocyte interleukin 1α and βmRNA. Keratinocyte epidermal cell-derived thymocyte-activating factor is identical to interleukin 1. J Exp Med 164:2095–2100, 1986
14. Luger TA, Stadler BM, Luger BM, Sztein MB, Schmidt JA, Hawley-Nelson P, Grabner G, Oppenheim JJ: Characteristics of an epidermal cell thymocyte-activating factor (ETAF) produced by human epidermal cells and a human squamous cell carcinoma cell line. J Invest Dermatol 81:187–193, 1983
15. Bell TV, Harley CB, Stetsko D, Sauder DN: Expression of mRNA homologous to interleukin-1 in human epidermal cells. J Invest Dermatol, in press
16. Lyons MC, Towers B (eds): Galen on Anatomical Procedures. The Later Books. New York, Cambridge University Press, 1962
17. Bach J-F, Dardenne M, Pleau J-M, Bach M-A: Isolation, biochemical characteristics, and biological activity of a circulating thymic hormone in the mouse and in the human. Ann N Y Acad Sci 249:186–210, 1975
18. Hirokawa K: The thymus and aging. In Makinodan T, Yunis E (eds): Immunology and Aging. New York, Plenum Medical Book Co., 1977, pp 51–72
19. Rheinwald JG, Green H: Serial cultivation of strains of human epidermal keratinocytes. The formation of keratinizing colonies from single cells. Cell 6:331–344, 1975
20. Maciag T, Nemore RE, Weinstein R, Gilchrest BA: An endocrine approach to the control of epidermal growth: serum-free cultivation of human keratinocytes *in vitro*. Science 211:1452–1454, 1981
21. Gilchrest BA, Marshall WL, Karassik RL, Weinstein R, Maciag T: Characterization and partial purification of keratinocyte growth factor from the hypothalamus. J Cell Physiol 120:377–383, 1984
22. Gilchrest BA: In vitro assessment of keratinocyte aging. J Invest Dermatol 81:184S-189S, 1983
23. Gilchrest BA, Calhoun JK, Maciag T: Attachment and growth of human keratinocytes in a serum-free environment. J Cell Physiol 112:197–206, 1982
24. Stanulis-Praeger BM, Gilchrest BA: Growth factor responsiveness declines during adulthood for human skin-derived cells. Mech Ageing Dev 35:185–198, 1986
25. Ueda K, Hayaishi O: ADP ribosylation. Annu Rev Biochem 54:73–100, 1985
26. Green H: Cyclic AMP in relation to proliferation of the epidermal cell. A new view. Cell 15:801–811, 1978
27. Low TLK, Goldstein AL: Thymic hormones: an overview. Methods Enzymol 116:213–291, 1985
28. Schlesinger DH, Goldstein G: The amino acid sequence of thymopoietin II. Cell 5:361–365, 1975
29. Mizel SB: Studies on the purification and structure-function relationship of murine lymphocyte activating factor (interleukin 1). Mol Immunol 17:571–577 1980
30. Oppenheim JJ, Mizel SB, Meltzer MS: Biological effects of lymphocyte and macrophage-derived mitogenic "amplification" factors. In Cohen S, Pick E, Oppenheim JJ (eds): Biology of the Lymphokines. New York, Academic Press, 1979, pp 291–328
31. Hooper JA, McDaniel MC, Thurman GB, Cohen GH, Schulof RS, Goldstein AL: The purification and properties of bovine thymosin. Ann NY Acad Sci 249:125–127, 1975
32. Low TLK, Goldstein AL: The chemistry and biology of thymosin. II, Amino acid sequence analysis of thymosin α1 and polypeptide β1. J Biol Chem 254:987–995, 1979
33. Low TLK, Goldstein AL: Chemical characterization of thymosin β4. J Biol Chem 257:1000–1006, 1982
34. Dardenne M, Pleau J-M, Nabarra B, Lefrancier P, Derrien M, Choay J, Bach JF: Contribution of zinc and other metals to the biological activity of the serum thymic factor. Proc Natl Acad Sci USA 79:5370–5373, 1982
35. Le PT, Tuck DT, Dinarello CA, Haynes BF, Singer KH: Human thymic epithelial cells produce interleukin 1. J Immunol 138:2520–2526, 1987
36. Ristow H-J: A major factor contributing to epidermal proliferation in inflammatory skin diseases appears to be interleukin 1 or a related protein. Proc Natl Acad Sci USA 84:1940–1944, 1987
37. Sauder DN, Stanulis-Praeger BM, Gilchrest BA: Autocrine growth stimulation of human keratinocytes by epidermal cell-derived thymocyte activating factor: implications for skin aging. Arch Dermatol Res (in press)
38. Oppenheim JJ, Stadler BM, Siraganian RP, Mage M, Mathieson, B: Lymphokines: their role in lymphocyte responses. Properties of interleukin 1. Fed Proc 41:257–262, 1982
39. De Chiara TM, Young D, Semionow R, Stern AS, Batula-Bernardo C, Fiedler-Nagy C, Kaffka KL, Kilian PL, Yamazaki S, Mizel SB, Lomedico PT: Structure-function analysis of murine interleukin 1: biologically active polypeptides are at least 127 amino acids long and are derived from the carboxyl terminus of a 270-amino acid precursor. Proc Natl Acad Sci USA 83:8303–8307, 1986
40. Soder O: Thymocyte growth factor: a progression growth factor for cycling immature cortical thymocytes. Comp Immun Microbiol Infect Dis 8:235–246, 1985
41. Zatz MM, Oliver J, Samuels C, Skotnicki AB, Sztein MB, Goldstein AL: Thymosin increases production of T-cell growth factor by normal human peripheral blood lymphocytes. Proc Natl Acad Sci USA 81:2882–2885, 1984
42. Soder O, Ernstrom U: Purification to apparent homogeneity of a thymocyte specific growth factor from calf thymus. Biochem Biophys Res Commun 120:420–426, 1984

43. Goldstein AL, Thurman GB, Low TLK, Rossio JL, Trivers GE: Hormonal influences on the reticuloendothelial system: current status of the role of thymosin in the regulation and modulation of immunity. J Reticuloendothel Soc 23:253–266, 1978

44. Luckey TD: Thymic hormones. Baltimore, University Park Press, 1973

45. Naylor PH, Goldstein AL: Thymosin: cyclic nucleotides and T-cell differentiation. Life Sci 25:301–309, 1979

46. Marcelo CL, Tomich J: Cyclic AMP, glucocorticoid, and retinoid modulation of in vitro keratinocyte growth. J Invest Dermatol 81:64s–68s, 1983

47. Okada N, Kitano Y, Ichihara K: Effects of cholera toxin on proliferation of cultured human keratinocytes in relation to intracellular cyclic AMP levels. J Invest Dermatol 79:42–47, 1982

48. Ohno T: Strict relationship between dialyzed serum concentration and cellular lifespan *in vitro*. Mech Ageing Dev 11:179–186, 1979

49. Phillips PD, Kaji K, Cristofalo VJ: Progressive loss of the proliferative response of senescing WI-38 cells to platelet-derived growth factors, epidermal growth factor, insulin, transferrin, and dexamethasone. J Gerontol 36:11–17, 1984

50. Harley CB, Goldstein S, Posner BI, Guyda H: Decreased sensitivity of old and progeric human fibroblasts to a preparation of factors with insulin like activity. J Clin Invest 68:988–994, 1981

51. Plisko A, Gilchrest BA: Growth factor responsiveness of cultured human fibroblasts declines with age. J Gerontol 38:513–518, 1983