found that both our class II and class I MHC alloreactive γδ T cell lines failed to respond to heat-shocked syngeneic APCs, to crude mycobacterial sonicates rich in hsp's, or to partially purified extracts of 65-, 70-, and 12-kD hsp's derived from mycobacterium tuberculosis (*24*). Therefore, mycobacterial hsp-specific TCRγδ T cells and MHC-specific TCRγδ T cells may constitute distinct functional cell subsets.

Both the TCRγδ and TCRαβ T cell lines we used were derived from athymic mice and thus they represent examples of functional extrathymic T cell maturation for class II MHC molecule recognition. One striking feature of the specificity pattern of the class II MHC alloreactive TCRγδ-expressing T cells is their cross-reactivity for the products of multiple distinct I-E alleles. This is in contrast to the unique specificity of the TCRαβ LBK4 cells for the E^k molecule expressed on the immunizing B10.BR APC. The class I MHC–specific TCRγδ cell line we described earlier also had a broad pattern of cross-reactivity for multiple allogeneic class I MHC antigens (*3, 5*). It is possible that TCRγδ in general may be specific for distinct determinants on MHC molecules less polymorphic than those recognized by TCRαβ.

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Neonatal Thymectomy Results in a Repertoire Enriched in T Cells Deleted in Adult Thymus

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In B6AF1 mice, T lymphocytes that use the $V_\beta11$-positive (and not $V_\beta6$-positive or $V_\beta8$-positive) segment in their receptor for antigen are greatly reduced in the thymus and peripheral lymphoid tissues, most likely as a result of clonal deletion. The relative number of $V_\beta11$-positive cells in adult lymph nodes was ten times as high in B6AF1 mice thymectomized 1 to 4 days after birth as in normal mice. Moreover, for the first 10 days of life of B6AF1 mice, mature $V_\beta11$-positive T cells were readily detected in the thymus and spleen. Thus neonatal thymectomy results in the maintenance of the receptor repertoire of early postnatal life, and this correlates with the subsequent development of organ-specific autoimmune diseases.

ONE MECHANISM OF SELF TOLERance involves the deletion of T cell clones in the thymus. Thus T cells with antigen receptors of specific V_β families do not mature intrathymically in mice expressing the major histocompatibility complex (MHC) molecule I-E (*1–3*), or the minor lymphocyte stimulatory (Mls) antigens (*4–6*). The elimination of such T cell clones appears to involve intrathymic interaction of T cell receptors (TCR) with MHC antigen on thymic stromal cells (*7–9*). This seminal concept implies that autoreactive T cells that encounter self antigens bound to MHC intrathymically would be similarly deleted. We now show that mice thymectomized soon after birth (days 1 to 4) contain in their peripheral lymphoid tissue T cells with TCR that would normally be deleted in the thymus. This finding indicates that clonal deletion is imperfect or leaky so that when thymopoiesis is ablated, autoreactive clones that emigrate from the neonatal thymus can expand in the periphery and become prominent. Such thymectomized mice exhibit autoimmune traits. Thus some autoimmune reactions may result from imperfect clonal deletion.

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A/J or (C57BL/6 × A/J)F_1 (B6AF1) mice thymectomized between day 1 and 4 after birth, usually on the third day (D3TX), develop autoimmune disease in the ovary, testis, thyroid, and stomach (*10, 11*). The pathogenesis of the disease is unknown. Both B6AF1 and A/J mice express I-E molecules; such I-E$^+$ mice delete mature thymocytes and peripheral T cells with antigen receptors expressing $V_\beta 11$ but not those expressing $V_\beta 6$ or $V_\beta 8$ (*12*). To determine the effect of D3TX on the adult T cell repertoire, we compared the proportion of T cells expressing αβ TCRs, $V_\beta 11$, $V_\beta 6$, and $V_\beta 8$ in D3TX and normal adult B6AF1 mice (Table 1 and Fig. 1). The lymph nodes of D3TX and normal mice had similar percentages of $V_\beta 6^+$ and of $V_\beta 8^+$ cells per αβ T cell. In contrast, the percentage of $V_\beta 11^+$ T cells in the lymph nodes of D3TX mice was 10.4-fold as high as that of normal mice. A selective enrichment of $V_\beta 11^+$ T cells was also found in the spleen of D3TX mice. No significant difference was found in $V_\beta 11^+$, $V_\beta 6^+$, or $V_\beta 8^+$ lymph node T cells from the normal and D3TX mice when compared by two-color flow cytometry for CD4 and CD8 antigen expression. Specifically, there were no detectable $CD4^-$ $CD8^-$ $V_\beta 11^+$ T cells in the D3TX mice (*13*).

Since the T cell repertoire of a D3TX mouse should be derived from the repertoire of normal 3-day-old mice, we investigated the thymus of normal neonatal B6AF1 mice for evidence of clonal deletion of T cells bearing TCRs expressing $V_\beta 11^+$ and $V_\beta 6^+$ segments (Table 2 and Fig. 2, A to D). The percentages of thymocytes expressing a high density of αβ TCRs and $V_\beta 6$ were similar in mice ranging in age from 3 days to adulthood, an indication that these cells were not deleted (*12*). In contrast, although $V_\beta 11^+$ thymocytes with high TCR expression were deleted in the thymus of 2-week-old (1.5%) and adult (0.6%) mice, they were readily detectable in the thymus of the B6AF1 mice on day 1 (7.0%) and on day 3 (7.9%). Moreover, during the first neonatal week, the percentage of $V_\beta 11^+$ T cells in the B6AF1 thymus was comparable to the percentage of $V_\beta 11^+$ cells in the thymus of I-E^- (C57BL/6) mice (Table 2). Therefore, T cells that are normally deleted in the adult thymus are not deleted during the first week of life.

We next studied the expression of TCRs in the spleens of neonatal B6AF1 mice. An unexpectedly high proportion of αβ T cells

Table 1. Quantitation of V_β TCR^+ cells in lymph nodes and spleens of normal and day 3 thymectomized adult B6AF1 mice. The T cells expressing the $V_\beta 6$, $V_\beta 8$ (8.1 ± 8.2) or $V_\beta 11$ segment were calculated as a percentage of αβ TCR-positive T cells (mean ± SD). The data from one experiment are shown in Fig. 1. Pathogen-free B6AF1 and C57BL/6 mice of our mouse colony were derived from mice purchased from the Jackson Laboratory (Bar Harbor, Maine) and their care was in accordance with our institutional guideline. Three-day-old female mice anesthetized by hypothermia were thymectomized by the suction technique (*11*). Suspensions of unseparated lymph node and spleen cells from D3TX and normal mice, aged 4 months, were processed for flow cytometry. Red blood cells were lysed by 0.9% ammonium chloride. Cells were incubated at 4°C for 20 min with fluorescein isothiocyanate (FITC)–labeled monoclonal antibody H57-597 to αβ TCRs (*20*), monoclonal antibody RR-47 to $V_\beta 6$ (*21*), or monoclonal antibody RR-315 to $V_\beta 11$ (*12*). Incubation with monoclonal antibody KJ-16 to $V_\beta 8$ (8.1 ± 8.2) (*22*) was done at 37°C for 20 min. After being washed in phosphate-buffered saline with 0.5% bovine serum albumin and 0.1% sodium azide, the cells were incubated with FITC-labeled goat antibody to rat immunoglobulin G (Tago, Burlingame, California) for 20 min. The cells were washed and then analyzed on a FACScan flow cytometer (Becton Dickinson, Mountain View, California). Nonviable cells were excluded by propidium iodide, and 20,000 cells per sample were analyzed. Background cell counts (less than 1%) due to staining by FITC-labeled antibody to rat immunoglobulin G alone were subtracted from experimental cell counts. Three experiments were conducted per category.

Cell	Mouse	$V_\beta 11$		$V_\beta 6$		$V_\beta 8$	
		Cells (%)	D3TX: normal	Cells (%)	D3TX: normal	Cells (%)	D3TX: normal
Lymph node	Normal	0.88 ± 0.2*		10.0 ± 0.7		23.3 ± 0.9	
	D3TX	9.14 ± 2.5*	10.4	15.7 ± 2.7	1.4	22.0 ± 0.6	1.0
Spleen	Normal	3.60 ± 0.4		13.7 ± 0.4		27.3 ± 4.0	
	D3TX	15.00 ± 3.3	4.2	24.3 ± 3.64	1.7	30.8 ± 7.5	1.1

*Significantly different by Student's *t* test ($P = 0.005$).

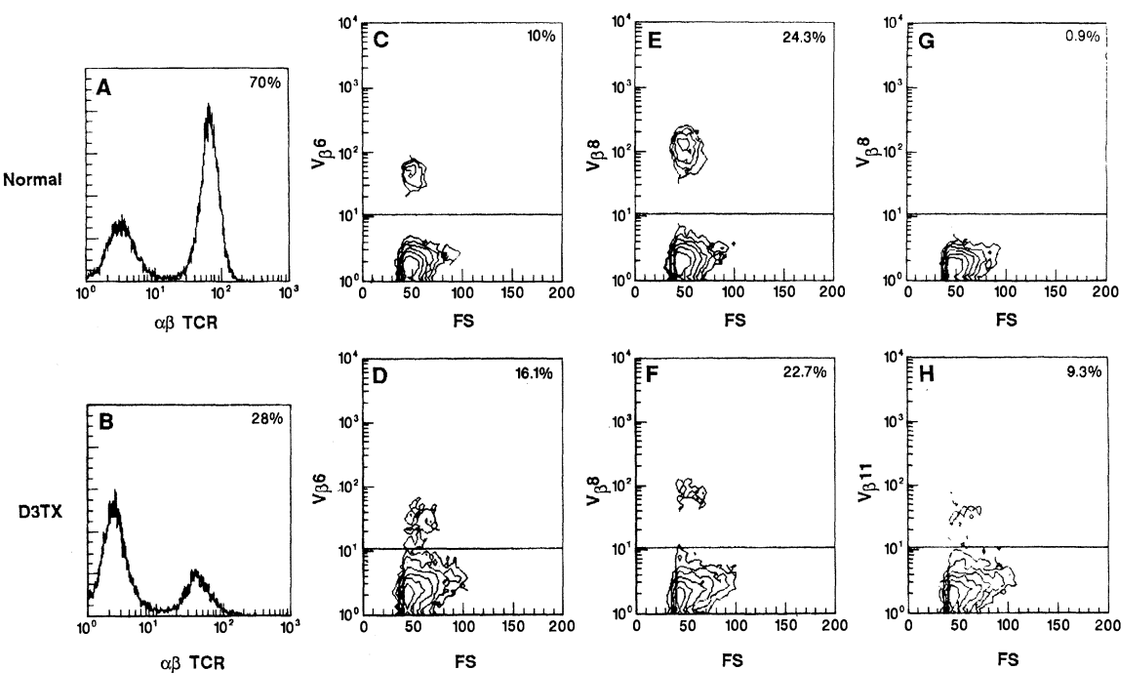

Fig. 1. Quantitation of cell surface expression of αβ TCRs and V_β-specific TCR^+ T cells in lymph nodes of 4-month-old D3TX and normal mice. Lymph node cells were processed for flow cytometry as described for Table 1. (**A**, **C**, **E**, **G**) Data for normal mice; (**B**, **D**, **F**, **H**) data for D3TX mice. The total αβ TCR^+ T cells of D3TX mice are reduced to 40% of normal (A and B). The percentages of $V_\beta 6^+$ (C and D) and of $V_\beta 8^+$ (8.1 + 8.2) (E and F) T cells in D3TX and normal mice are similar. In contrast, D3TX mice have ten times more $V_\beta 11^+$ T cells than normal mice (G and H). Fluorescence intensity is analyzed on log scale on the horizontal axis for (A) and (B); and on vertical axis for (C) to (H) (FS, forward scatter analysis).

expressing $V_\beta11^+$ was found in the spleens of 3-day-old mice; in three experiments, it ranged from 21% to 46% with a mean of 29.4% (Table 2 and Fig. 2, E to H). By day 5, the number was 10.5%. After 2 weeks, concomitant with the increase in αβ T cells, the proportion of $V_\beta11^+$ T cells was reduced to 2.3%, suggesting that $V_\beta11^+$ T cells were diluted out by T cells (including $V_\beta6^+$ T cells) that could mature in an I-E^+ thymus.

We have shown that the thymus of B6AF1 mice in the first week of life permits the maturation of $V_\beta11^+$ T cells that normally fail to mature in an I-E^+ adult thymus. In the first 2 weeks, $V_\beta11^+$ T cells were also readily detectable in the spleen. In normal I-E^+ B6AF1 mice, the $V_\beta11^+$ T cells in the peripheral lymphoid organs were diluted out by T cells that mature in the adult thymus so that fewer than 1% of the αβ T cells in the adult lymph nodes were $V_\beta11^+$. The findings regarding $V_\beta11^+$ T cells are not unique to $V_\beta11^+$ T cells. We have observed changes that parallel those of $V_\beta11^+$ T cells in the B6AF1 mice for $V_\beta3^+$ T cells (*13*). $V_\beta3^+$ T cells are deleted in Mls^{c+} mice (*6*); A/J mice are Mls^{c+}, and Mls antigen expression is a codominant trait (*14*).

It is possible that the neonatal thymus is functionally incompetent with respect to clonal deletion at this stage of very vigorous thymopoiesis. Alternatively, an interesting

Table 2. Quantitation of αβ and V_β TCR^+ thymocytes and spleen T cells in normal B6AF1 and C57BL/6 mice. Thymocytes and spleen cells from three 3-day-old normal mice were pooled and those from two 7-day-old mice were pooled in each experiment. Data for αβ T cells are expressed as the percentage of total thymocytes or the percentage of nucleated spleen cells; data for $V_\beta6^+$ and $V_\beta11^+$ T cells are expressed as percentage of αβ T cells. Processing of cells for flow cytometry and statistical analysis of data were as described for Table 1. In flow cytometric analysis of thymocytes, the cells that segregated into three distinct populations were gated: negative cells, cells expressing low quantity of TCR (immature thymocytes), and cells expressing high quantity of TCR (mature thymocytes) (see Fig. 2). For thymocytes that are not deleted (for example, those containing αβ TCRs and $V_\beta6^+$ thymocytes) the detectable levels of mature thymocytes did not show significant change with increasing age of the mice. $V_\beta11^+$ mature B6AF1 thymocytes are initially detectable but after day 7 the number diminishes rapidly, a finding indicative of absence of intrathymic maturation, or clonal deletion. In contrast, $V_\beta11^+$ thymocytes of I-E^- C57BL/6 mice show no evidence of deletion. Data are means ± SD from three experiments. Those from one experiment are shown in Fig. 2. ND, not determined.

Cell	Mouse	TCR	TCR quantity	Cells (%) Day 3	Day 5	Day 7	Day 14	Adult
Thymus	B6AF1	αβ	High	14.0 ± 2.2	8.9 ± 1.7	7.5 ± 0.4	10.6 ± 2.1	14.2 ± 3.4
			Low	40.2 ± 2.4	41.7 ± 1.8	40.6 ± 1.4	40.4 ± 3.4	44.9 ± 2.3
		Vβ6	High	10.6 ± 2.6	5.0 ± 0.9	7.8 ± 0.1	8.8 ± 4.0	5.0 ± 1.2
			Low	8.3 ± 0.8	7.6 ± 1.2	7.4 ± 1.7	6.9 ± 0.6	6.1 ± 1.2
		Vβ11	High	7.9 ± 3.8*	4.5 ± 1.7	5.2 ± 2.8	1.5 ± 1.1	0.6 ± 0.6*
			Low	5.5 ± 1.1*	6.6 ± 2.6	7.1 ± 1.9	6.0 ± 1.7	5.4 ± 2.2*
	C57BL/6	αβ	High	7.1 ± 5.1	ND	6.7 ± 1.7	ND	11.9 ± 0.2
			Low	48.6 ± 3.1	ND	38.0 ± 2.4	ND	45.0 ± 4.6
		Vβ6	High	8.6 ± 3.2	ND	10.2 ± 1.0	ND	5.0 ± 0.9
			Low	7.3 ± 1.0	ND	5.5 ± 1.4	ND	5.1 ± 1.1
		Vβ11	High	5.9 ± 1.0	ND	5.7 ± 1.9	ND	4.7 ± 0.1
			Low	7.1 ± 0.9	ND	5.5 ± 1.5	ND	6.6 ± 1.4
Spleen	B6AF1	αβ		6.6 ± 2.9	7.7 ± 2.5	7.1 ± 1.6	9.1 ± 2.0	32.3 ± 2.9
		Vβ6		12.0 ± 3.2	11.2 ± 2.8	11.2 ± 1.2	9.4 ± 3.2	12.4 ± 2.0
		Vβ11		29.4 ± 13.4†	10.5 ± 0.6	11.1 ± 0.8	9.8 ± 2.3	2.3 ± 1.6†

*The number of $V_\beta11$ thymocytes from 3-day-old and adult mice was significantly different ($P = 0.02$). There was no significant difference between adult and 7-day-old mice or adult and 14-day-old mice for $V_\beta11$ thymocytes. †Significantly different by Student's t test ($P = 0.02$). There was no significant difference between the numbers of $V_\beta6^+$ T cells in spleens of 3-day-old and adult mice.

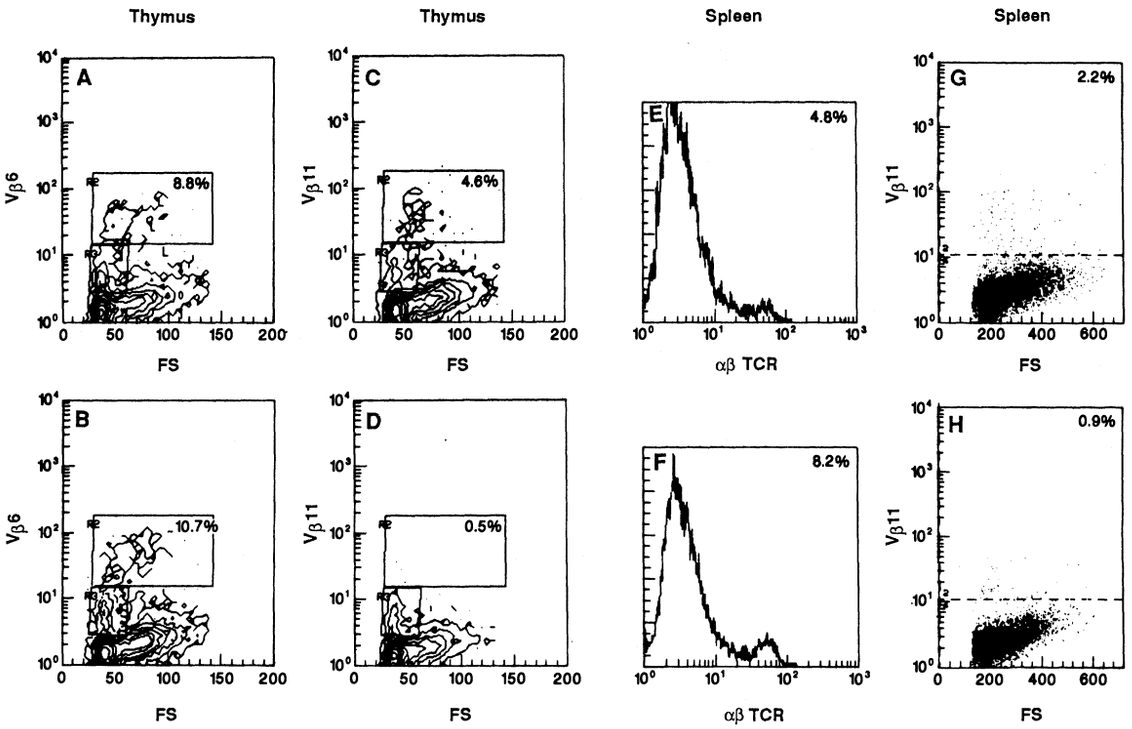

Fig. 2. Quantitation of αβ TCRs and V_β-specific TCRs on thymocytes and spleen cells of normal young B6AF1 mice. Thymocytes pooled from (**A** and **C**) 3-day-old and (**B** and **D**) 14-day-old female B6AF1 mice were analyzed by flow cytometry for $V_\beta6^+$ (A and B) and $V_\beta11^+$ (C and D) T cells. Comparable percentages of mature thymocytes with bright staining $V_\beta6^+$ TCRs are detected in mice of both ages. In contrast, mature thymocytes with bright staining $V_\beta11^+$ TCR are detected in 3-day-old but not in 14-day-old mice. Spleen cells from 3-day-old (**E** and **G**) and 7-day-old (**F** and **H**) mice were analyzed for αβ TCR^+ (E and F) and for $V_\beta11^+$ (G and H) T cells. In the 3-day-old spleen, 4.8% of the cells are αβ TCR^+ (E) and 2.2% of the spleen cells are $V_\beta11^+$ (G), thus 46% of αβ TCR^+ cells are $V_\beta11^+$. On day 7, the percentage of αβ TCR^+ T cells has doubled (F) and the percentage of $V_\beta11^+$ T cells is reduced to 0.9% (H), hence the $V_\beta11^+$ T cells per αβ TCR^+ T cells are reduced to 11%. Fluorescence intensity is analyzed on a log scale. (FS, forward scatter analysis.)

and provocative speculation is that the elimination of $V_\beta 11^+$ T cells by I-E may require engagement of their TCR with a self peptide in association with self I-E (*15*), and the absence of deletion may reflect an absence of the putative self peptide during the neonatal week. Thus, the absence of a self peptide in the thymus either permanently or for a limited time would result in the occurrence of autoreactive T cells in peripheral organs. Regardless of why $V_\beta 11^+$ T cells are not deleted in I-E^+ neonatal mice, the finding of maturation of these autoreactive T cells and their emigration to the spleen provides a plausible explanation for the paradox of clonal deletion and the existence of autoreactive T cells in the normal adult peripheral lymphoid organs.

If T cells that are normally deleted in the thymus can leave the thymus during the neonatal period, why do they not cause autoimmune diseases in the normal mice? Although we have suggested that these cells persist in the normal adults, we have not yet ruled out the possibility that they are subsequently deleted, particularly as they recirculate through the medulla of a more mature thymus. Alternatively, these nondeleted T cells may persist, but tolerance mechanisms other than deletion (so-called peripheral tolerance) normally prevent them from being activated by self antigens (*16*). This is suggested by the finding that the sizes of the $V_\beta 11^+$ T cells in the D3TX mice were in the range of nonactivated small lymphocytes (Fig. 1H). That autoimmune diseases elicited by D3TX can be prevented by normal spleen T cells also supports this possibility (*10*).

The representation of large numbers of $V_\beta 11^+$ T cells among the αβ T cells in the spleens of 3-day-old mice was unexpected. The finding is important in the consideration of autoimmunity that results from D3TX. Since thymectomy eliminates the source of T cells, D3TX should fix the T cell repertoire of the mice to one enriched in $V_\beta 11^+$ (and $V_\beta 3^+$) T cells. This is indeed the case since the $V_\beta 11^+$ T cells in the lymph node and spleen of adult D3TX mice are significantly enriched in relation to those T cells that can normally mature in the thymus. In our preliminary study, $V_\beta 3^+$ T cells also represented a high percentage of the αβ T cells in B6AF1 mice after D3TX (*13*). In addition, D3TX mice had 40% of the normal number of αβ T cells (Fig. 1, A and B). D3TX, therefore, markedly skews the T cell repertoire of adult mice to one enriched in those T cells that are normally deleted in the adult thymus.

The finding that D3TX mice develop a high incidence of organ-specific autoimmune diseases supports the notion that T cells with specific V_β segments have a direct role in organ-specific autoimmunity. In this regard, mice treated with cyclosporine A also have mature, autoreactive T cells in the thymus (*3, 17*), and they develop autoimmune diseases similar to those of D3TX mice (*18*). Furthermore, in mice of the H-2^u haplotype, experimental autoimmune encephalomyelitis induced by immunization with the peptide 1-11 of myelin basic protein is associated with the function of $V_\beta 8$-specific T cells (*19*). Further studies of mice with autoimmune disease should help to determine whether clonal deletion is the mechanism of tolerance to organ-specific autoantigens and to further clarify the role of the undeleted autoreactive clones in autoimmune disease pathogenesis.

Note added in proof: Schneider *et al.* (*23*) also observed that $V_\beta 6^+$ cells were not deleted in the neonatal Mls^{a+} mice. Their findings therefore supported our results, and were an inspiration to this study.

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Human KGF Is FGF-Related with Properties of a Paracrine Effector of Epithelial Cell Growth

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Keratinocyte growth factor (KGF) is a human mitogen that is specific for epithelial cells. The complementary DNA sequence of KGF demonstrates that it is a member of the fibroblast growth factor family. The KGF transcript was present in stromal cells derived from epithelial tissues. By comparison with the expression of other epithelial cell mitogens, only KGF, among known human growth factors, has the properties of a stromal mediator of epithelial cell proliferation.

GROWTH FACTORS THAT ARE SECRETED by certain cells and act on nearby responsive cells function in the development of multicellular organisms (*1*). Such paracrine-acting growth factors also appear to participate in the renewal of normal hematopoietic cell populations (*2*). Epithelial cells that line the skin and gastrointestinal (GI) tract also turnover rapidly. Although there are several growth factors that include epithelial cells among their known targets, none has yet been established as important in proliferation of normal epthelial tissues. We recently purified a growth factor, keratinocyte growth factor (KGF), that is active on keratinocytes and appears to be specific for epithelial cells (*3*). We now describe the isolation of the cDNA for KGF and its possible role in epithelial cell growth and development.

Oligonucleotide probes were generated

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