cytosol, with the rest being completely imported. In fact, such a pathway might explain the dual targeting of fumarase in *Saccharomyces cerevisiae* (*23*).

Finally, our data suggest that the mitochondrial import machinery contains a passive import channel formed by components in both membranes. This import channel accommodates unfolded polypeptide chains, but components of the channel interact only weakly with mature segments of translocating chains as compared to mtHsp70.

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9. Fusion protein pSu9(1–112)-DHFR was prone to aggregation in translation lysates. This was reflected by its low import efficiency of approximately 20% of input. Import efficiencies of pSu9(1–86)-DHFR and pSu9(1–94)-DHFR were between 70 to 80% of input. Import efficiency of Su9(1–112)-DHFR increased by 50% in the presence of Mtx (Fig. 1). This was probably due to prevention of Su9(1–112)-DHFR aggregation through stabilization of the DHFR domain by Mtx (Fig. 1). However, the DHFR domain on all the pSu9(1–112)-DHFR molecules was not completely folded. This prevented a complete block in processing to the m form from occurring in the presence of Mtx (Fig. 1). A band corresponding in size to the m form of Su9-DHFR was detected in the supernatants of reaction mixtures (Fig. 1). This band was not generated because of import into the matrix. It was present in translation lysates (*8*), did not increase during incubations, and is likely to have resulted from translation initiation at an internal methionine.
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17. The efficiency of pSu9(1–94)-DHFR import into mitoplasts was ~30% lower than that of import into mitochondria. In addition, a large portion of the m form remained sensitive to digestion by protease (Fig. 3). Thus, a portion of the m form was in a conformation in which it spanned both membranes. In mitoplasts, at both high and low matrix ATP, a slightly higher level of complex formation between Su9(1–94)-DHFR and mtHsp70 was detected as compared to that in mitochondria (Fig. 3). This difference apparently results from interaction of mtHsp70 with the m form that was not completely imported into the matrix.
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Modulation of Epithelial Cell Growth by Intraepithelial γδ T Cells

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The role played in immune surveillance by γδ T cells residing in various epithelia has not been clear. It is shown here that activated γδ T cells obtained from skin and intestine express the epithelial cell mitogen keratinocyte growth factor (KGF). In contrast, intraepithelial αβ T cells, as well as all lymphoid αβ and γδ T cell populations tested, did not produce KGF or promote the growth of cultured epithelial cells. These results suggest that intraepithelial γδ T cells function in surveillance and in repair of damaged epithelial tissues.

Thy-1$^+$ dendritic epidermal T cells (DETCs) (*1–3*) expressing the invariant $V_\gamma3$ $V_\delta1$ T cell receptor (TCR) (*4*, *5*) are found exclusively in the epidermis of the adult mouse (*6*), where they exist in intimate contact with keratinocytes. The expression of a monoclonal antigen recognition structure by DETCs contrasts with the large TCR repertoire of nonepithelial αβ and γδ T cells. This lack of diversity suggests that DETCs have a limited ligand recognition capacity and may have a different role in immunological surveillance from that of αβ T cells (*7*). We have shown that DETCs recognize self-antigen presented by cultured or stressed keratinocytes in an apparently non–major histocompatibility complex (MHC)–restricted fashion (*8*). The existence of a functional link between two cell types normally in close physical contact led us to investigate the possibility that this connection extends to the expression by DETCs of growth factors acting on keratinocytes.

Balb/MK keratinocytes are not viable when grown under defined serum-free conditions, except when supplemented with either exogenous KGF, acidic fibroblast growth factor (FGF), basic FGF, epidermal growth factor (EGF), or insulin growth factor–1 (Fig. 1A) (*9*, *10*). Other factors alone or in combination could conceivably support the proliferation of Balb/MK cells (*10*). We used the growth factor dependence of the Balb/MK line to devise an experimental system with membrane filters that allowed diffusible factors secreted by the 7-17 DETC line (*11*) to reach Balb/MK cells while preventing direct cell contact.

In transfilter experiments, we assessed the ability of 7-17 cells to produce a cytokine that would promote the proliferation of Balb/MK cells. The results of these experiments demonstrated that 7-17 cells, as well as three other DETC lines (*12*), release a soluble factor that can sustain the clonal growth of Balb/MK cells (Fig. 1, B and C). Stimulation of 7-17 cells beforehand with mitogen concanavalin A (Con A) potentiated this activity (Fig. 1D). In contrast, T cell lines bearing αβ receptors or γδ receptors other than $V_\gamma3$ $V_\delta1$ did not prevent Balb/MK cell death under identical conditions (Fig. 1D).

The ability of DETCs to inducibly secrete a mitogenic factor that targets keratinocytes represents a possible function for a T cell population expressing a monomorphic TCR that exclusively recognizes stressed or otherwise damaged keratinocytes. Consideration of cytokines with the potential to mediate this effect led us to investigate KGF as the most likely candidate because of its specificity toward keratinocytes (*13*, *14*).

Hybridization of a human KGF comple-

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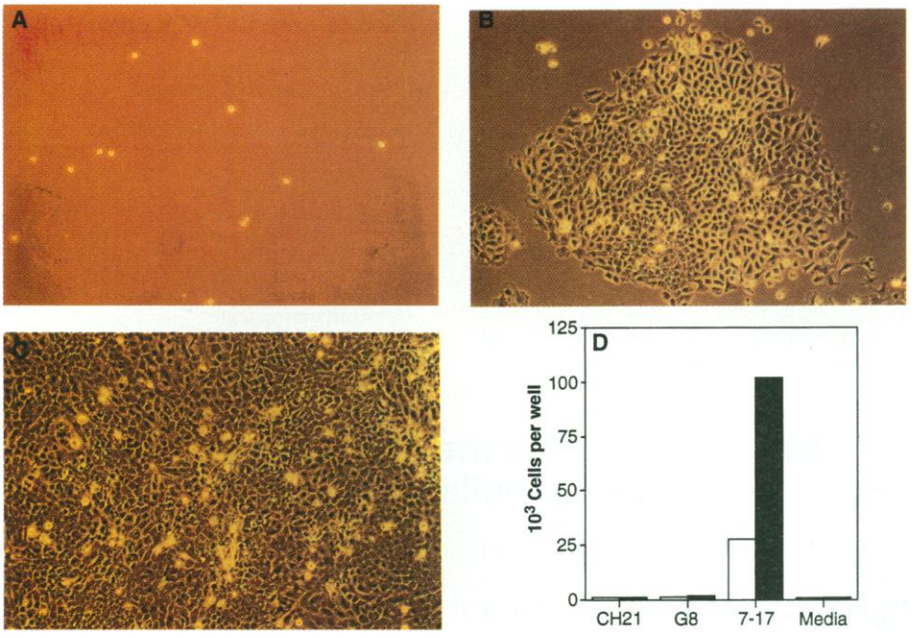

Fig. 1. Growth-promoting effect of DETC 7-17 cells on Balb/MK keratinocytes (*26*). Phase-contrast photomicrographs of Balb/MK keratinocytes grown for 12 days (**A**) in the absence of 7-17 cells, (**B**) after 5 days of co-culture to show individual colonies, and (**C**) after 12 days of co-culture to show confluence. Original magnification, ×100. (**D**) Comparison of the keratinocyte growth-promoting activity of the DETC 7-17 line, the αβ CH21 line, and the γδ G8 line with (filled bars) and without (open bars) stimulation first for 24 hours with Con A (2 μg/ml). Keratinocytes were counted after 12 days of co-culture. Data are representative of at least five experiments.

mentary DNA (cDNA) (*15*) to immobilized 7-17 RNA revealed a specific transcript of the appropriate length (Fig. 2A) (*16*). The KGF mRNA expressed by 7-17 cells was cloned by a reverse transcription–polymerase chain reaction (RT-PCR) with mouse KGF primers (*17*). The nucleotide and deduced amino acid sequences of the 585–base pair (bp) DNA fragment were identical to those for mouse KGF (*12*). Direct demonstration of KGF protein in media from cultured 7-17 DETCs was achieved with an antibody preparation capable of binding the NH_2-terminus of KGF (Fig. 2B). Conditioned medium from 7-17 cultures contained KGF (1 to 2 ng/ml), as determined by an enzyme-linked immunosorbent assay (ELISA) (*12*). We then used RT-PCR analysis to detect expression of KGF in various activated primary lymphoid T cell populations and T cell lines. We consistently failed to detect KGF expression by lymphoid αβ or γδ T lymphocytes, as shown for representative T cell lines with known TCR genes (Fig. 3A) and for primary T cell populations (Fig. 3B).

Freshly isolated, as well as $V_\gamma3^+$ sorted DETCs (*12*), did not produce KGF, suggesting that KGF expression is silenced in situ under normal conditions (Fig. 4A). In contrast, KGF expression was detected in cultured DETCs (Fig. 4A). Freshly isolated DETCs that are cultured for more than 24 hours slowly reach an activated state, as judged by interleukin-2 (IL-2) release (*18*). This in vitro situation is believed to reflect recognition of contaminating keratinocytes. Likewise, our initial observation that treatment with Con A augmented the mitogenic effects of 7-17 cells on keratinocytes led us to surmise that an increase in KGF expression follows DETC activation. The activation signal provided by mitogenic lectins is generally thought to be transduced by TCR aggregation (*19*). We tested whether KGF expression can be induced in primary DETCs as a result of signaling through the $V_\gamma3$ $V_\delta1$ TCR transduction cascade. Cross-linking of DETC TCRs with immobilized antibody to $V_\gamma3$ led to a relatively strong KGF response, whereas the combination of phorbol ester and ionomycin had a weaker ability to do so (Fig. 4A). The relative magnitude of DETC activation achieved with these specific treatments has been established (*20*) and correlates positively with KGF expression levels.

The antibody used in Fig. 2B did not neutralize the proliferation of keratinocytes cultured in the presence of KGF. Therefore, we sought an alternative approach to measure the relative contribution to Balb/MK survival of KGF derived from 7-17 cells. A peptide derived from a unique portion of the KGF receptor (KGFR) shown to specifically neutralize KGF was used (*21*). In our transfilter assay, this peptide abrogated the ability of 7-17 DETCs to stimulate the growth of Balb/MK cells (Fig. 4B). Heparin, also known to interfere with KGF function (*22*), had similar effects on the mitogenic activity of 7-17 DETCs (Fig. 4B). Results from the in vitro blocking assay provide evidence that KGF is the major keratinocyte mitogen released by DETCs. Taken together, the data presented support the

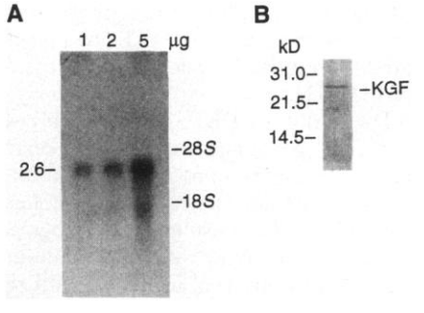

Fig. 2. Detection of mouse KGF expressed by DETC 7-17 cells. (**A**) Northern blot analysis of KGF mRNA expression (*16*). Total 7-17 RNA is indicated at the top of each lane in micrograms. (**B**) Immunoblot analysis of secreted KGF (*27*).

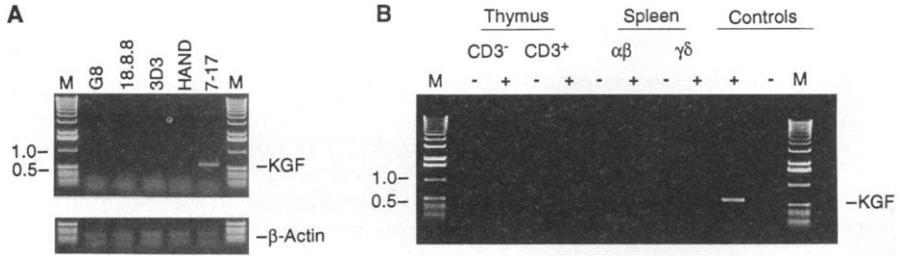

Fig. 3. KGF is not expressed by thymus or spleen T cells (*28*). (**A**) RT-PCR of representative cell lines G8 ($V_\gamma2$ $V_\alpha11$ TCR), 18.8.8 ($V_\gamma4$ $V_\delta6$ TCR), 3D3 ($V_\gamma1.1$ $V_\delta6$ TCR), HAND (αβ TCR), and 7-17 ($V_\gamma3$ $V_\delta1$ TCR) cultured 48 hours in the presence or absence of Con A (2 μg/ml) (only the mitogen-stimulated cultures are shown). KGF expression was not detected in any αβ or γδ TCR^+ cell lines tested with the exception of three DETC lines, including 7-17 (*12*). The RT-PCR of β-actin mRNA with the described primers (*29*) was performed as a control for the quantity and integrity of the RNA. (**B**) RT-PCR of isolated T cells cultured 48 hours in the presence (+) or absence (−) of Con A (2 μg/ml). M, a 1-kb DNA ladder. The positive control PCR used 7-17 cDNA and the negative control PCR used non–reverse transcribed RNA from 7-17 cells.

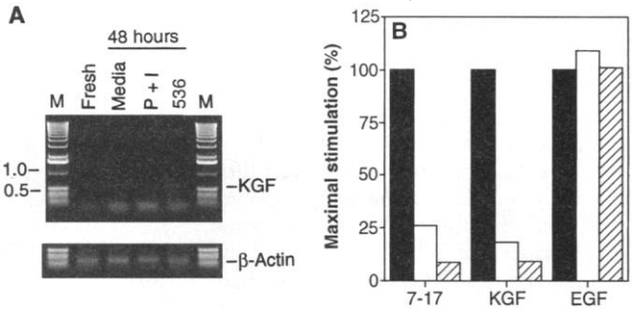

Fig. 4. (**A**) Evidence for the induction of KGF gene expression in DETCs. Epidermal cells (2×10^5) obtained as described (*8*) were immediately processed for RT-PCR or cultured for 48 hours, as indicated, before RNA isolation and RT-PCR (*16*). Phorbol ester and ionomycin (P + I) were used at 10 ng/ml and 1 μM, respectively; antibody 536 to $V_\gamma 3$ (*6*) was immobilized on plastic at a concentration of 1 mg/ml. RT-PCR of β-actin mRNA is shown as a control for the quantity and integrity of RNA. M, a 1-kb DNA ladder. (**B**) Inhibition of DETC-mediated proliferation of Balb MK cells by KGFR peptide (striped bars) and heparin (open bars) (*30*). The actual counts for Balb/MKs (black bars) co-cultured with 7-17 cells, recombinant KGF, recombinant EGF, or media were 4.6×10^5 cells, 4.4×10^5 cells, 1.02×10^6 cells, and 4×10^4 cells, respectively. Data are representative of at least three experiments.

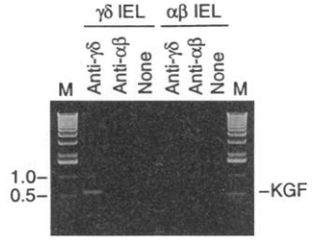

Fig. 5. KGF is inducibly expressed by γδ IELs but not αβ IELs (*31*). M, a 1-kb DNA ladder. Anti-γδ, antibody to pan γδ; anti-αβ, antibody to pan αβ.

view that KGF expression is a functional feature of DETC activation.

Reports indicate that KGF is a growth factor for several epithelia in vitro and in vivo (*23*, *24*). Like skin, the intestinal tract consists of rapidly renewing epithelial cells that form a barrier between the internal and external environments. Intestinal epithelial T cells (IELs) with γδ TCRs restricted in γ and δ segment use constitute a lymphocyte population of unknown function (*25*). We therefore investigated KGF expression by IELs. Activated, but not quiescent γδ IELs, expressed KGF (Fig. 5). In contrast, IELs with αβ TCRs did not express KGF under similar conditions (Fig. 5). These results suggest that KGF expression is a conserved feature of activated intraepithelial γδ T cell populations.

The restricted nature of the antigen receptor expressed by γδ T cells residing in epithelia made it seem likely that the function of these cells was distinct from other T cells. The ability of these γδ T cells to recognize and nurse injured epithelial cells may provide a local self-regulating mechanism to maintain the integrity of the epithelia.

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16. For Northern (RNA) blots, cytoplasmic RNA was extracted from cells and separated by electrophoresis in formaldehyde-containing agarose gels, as described [F. M. Ausubel *et al.*, Eds., *Current Protocols in Molecular Biology* (Greene, Toronto, Canada, 1988), pp. 4.1.2–4.1.6]. RNA was transferred to membranes (Hybond-N, Amersham) and processed for hybridization and detection, as directed by the manufacturer. The human KGF cDNA probe was labeled to a specific activity of at least 10^9 cpm/μg with α-^{32}P–labeled 2′-deoxyadenosine 5′-triphosphate (3000 Ci/mmol) by the random priming method. For RT-PCR, total RNA was extracted with TRIzol reagent (Gibco BRL), resuspended in 23.5 μl of distilled water, denatured at 70°C for 5 min in the presence of mouse KGF antisense primer 5′-CGGAATTCTTAGGTTATTGCCATAGGAAG-3′, and reverse transcribed at 37°C for 1 hour in a total volume of 30 μl of buffer containing 10 mM tris-HCl, pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl; 2.0 mM dithiothreitol (DTT), 0.25 mM deoxynucleotide triphosphates; 20 units of RNAsin (Promega) and 25 units of Moloney murine leukemia virus reverse transcriptase. DNA amplification was performed with sense primer 5′-CGGAATTCATGCGCAAATGGATACTGACACGG-3′, the antisense primer, and 10 μl of the reverse transcription reaction in 1× PCR reaction buffer (Boehringer Mannheim) containing 1 unit of Taq polymerase. Conditions for 30 cycles were 1 min at 94°C, 1 min at 60°C, and 1 min at 72°C. DNA amplification products were analyzed by agarose gel electrophoresis.
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26. The transwell co-culture system consisted of 2×10^4 Balb/MK cells seeded in six-well plates coated with fibronectin (1 μg/cm^2) and 1×10^5 7-17 cells added to Anocell 25 inserts (Whatman, Maidstone, England) in a total of 5 ml of Eagle's minimum essential medium (Biofluids, Rockville, MD) supplemented with transferrin (5 μg/ml), 10 nM Na_2SeO_3, 0.2 mM ethanolamine, insulin (5 μg/ml), and recombinant IL-2 (20 units/ml) (*10*).
27. A synthetic peptide encompassing mature KGF residues 1 through 13 was coupled to keyhole limpet hemocyanin, and we used the resulting conjugate to raise rabbit antibodies to KGF. The specificity of immune sera was determined by dot blot, protein immunoblot, and ELISA analysis with the use of ovalbumin-coupled peptide and a recombinant human KGF preparation (lot 11709, Upstate Biotechnology, Lake Placid, NY) as positive controls. KGF was semipurified and concentrated from 7-17 cell culture supernatant with heparin-Sepharose (Pharmacia) as described (*13*). Appropriate fractions were subjected to SDS–polyacrylamide gel electrophoresis (PAGE), transferred to polyvinylidene fluoride membranes (Millipore), and incubated with preimmune and selected immune sera. Specific complexes were revealed with an alkaline phosphatase detection system (Bio-Rad).
28. T cells from C57BL/6 mice were isolated from thymus and spleen tissues and incubated with monoclonal antibody (mAb) to CD3 (mAb 500A2) or to pan γδ (mAb GL3), followed by magnetic beads coated with antibodies to hamster immunoglobulin. Total RNA from positive and negative populations was extracted with TRIzol and subjected to RT-PCR as described (*16*). RNA integrity was evaluated after agarose gel electrophoresis and ethidium bromide staining of the samples.
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30. The transwell co-culture system consisted of 6×10^3 Balb/MK cells seeded in 24-well plates coated with fibronectin (1 μg/cm^2) and 5×10^4 7-17 cells added to Anocell 10 inserts in defined media, as described in (*26*). Media in control cultures lacking 7-17 cells were supplemented with a half-maximal dose of growth factor [recombinant KGF (1 ng/ml) or recombinant EGF (4 ng/ml; Biosource). Heparin (Sigma H-4898) (50 μg/ml) or KGFR peptide (50 μM, corresponding to KGFR residues 199 through 223) were added to all cultures as indicated. Keratinocytes were counted after 7 days of co-culture, and data were presented as percent of maximal stimulation.
31. IELs obtained as described (*25*) were incubated with fluorescein isothiocyanate (FITC)–conjugated antibody to pan αβ (mAb H57-597), biotinylated antibody to pan γδ (mAb GL3), and phycoerythrin (PE)–conjugated streptavidin. The αβ and γδ T cell populations were isolated with a flow cytometer (FACStar; Becton Dickinson). Sorted populations (>98% homogeneous) were cultured alone and in the presence of immobilized antibody to pan αβ or to pan γδ for 48 hours. RT-PCR was performed as described in (*16*).

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