A Role for TGFβ1 in Langerhans Cell Biology

Further Characterization of the Epidermal Langerhans Cell Defect in TGFβ1 Null Mice

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Abstract

Previous studies of TGFβ1 null (−/−) mice indicated that the epidermis was devoid of Langerhans cells (LC) and that the LC deficiency was not secondary to the inflammation that is the dominant feature of the −/− phenotype (Borkowski, T.A., J.J. Letterio, A.G. Farr, and M.C. Udey. 1996. *J. Exp. Med.* 184:2417–2422). Herein, we demonstrate that dendritic cells could be expanded from the bone marrow of −/− mice and littermate controls. Bone marrow from −/− mice also gave rise to LC after transfer into lethally irradiated recipients. Thus, the LC defect in TGFβ1 null mice does not result from an absolute deficiency in bone marrow precursors, and paracrine TGFβ1 production is sufficient for LC development. Several approaches were used to assess the suitability of −/− skin for LC localization. A survey revealed that although a number of cytokine mRNAs were expressed de novo, mRNAs encoding proinflammatory cytokines known to mobilize LC from epidermis (IL-1 and TNFα) were not strikingly overrepresented in −/− skin. In addition, bone marrow-derived LC populated full-thickness TGFβ1 null skin after engraftment onto BALB/c nu/nu recipients. Finally, the skin of transgenic mice expressing a truncated loricrin promoter-driven dominant-negative TGFβ type II receptor contained normal numbers of LC. Because TGFβ1 signaling in these mice is disrupted only in keratinocytes and the keratinocyte hyperproliferative component of the TGFβ1 −/− phenotype is reproduced, these results strongly suggest that the LC defect in TGFβ1 null mice is not due to an epidermal abnormality but reflects a requirement of murine LC (or their precursors) for TGFβ1. (*J. Clin. Invest.* 1997. 100:575–581.) Key words: Langerhans cell • dendritic cell • transforming growth factor β1 • growth factor • ontogeny

Introduction

Several cytokines play important roles in Langerhans cell (LC)[1] and dendritic cell (DC) biology. A central role for GM-CSF in LC/DC biology has been inferred from in vitro studies in which GM-CSF-containing conditioned media or recombinant GM-CSF has been demonstrated to support the growth, survival, and differentiation of murine as well as human DC (1–3). Selected in vivo studies demonstrating that local administration or overproduction of GM-CSF recruits LC/DC, or otherwise promotes accumulation of these rare cells, are consistent with this concept (4, 5). However, a very recent report suggests that the requirement of DC for GM-CSF in vivo is not absolute (6). A role for M-CSF (CSF-1) in LC physiology was initially suggested by results obtained from studies of the op/op (CSF-1-deficient) mouse (7). Subsequently, Takashima and coworkers determined that M-CSF (CSF-1) augments proliferation of murine LC lines (8). In vitro as well as in vivo studies indicate that proinflammatory cytokines such as IL-1 and TNFα promote LC survival, activation, and the initial stages of LC maturation, while T cell-derived IFNγ may be required for terminal maturation (9–13).

The pleiotropic cytokine TGFβ1 also has been implicated as an important regulator of LC and perhaps DC as well (14). We recently reported that the epidermis of TGFβ1 null (−/−) mice lacks LC. The deficiency was evident before the inflammatory syndrome that is characteristic of −/− mice was clinically apparent, and abrogation of the inflammatory syndrome via rapamycin administration for up to 7 wk did not reverse the LC component of the phenotype. Lymphoid DC were identified in lymph nodes of TGFβ1 null mice, but cell surface antigen expression was abnormal suggesting that the influence of TGFβ1 on cells of the DC lineage may not be restricted to skin. The present studies were initiated to further characterize the role of TGFβ1 in LC biology. We have taken advantage of the fact that murine LC precursors and the LC microenvironment (epidermis) can be studied independently in vivo, and the availability of a recently described transgenic mouse that selectively expresses a dominant-negative TGFβ type II receptor in epidermis (15) to localize the requirement for TGFβ1 to LC (or their precursors), and to begin to define the nature of the requirement.

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1. *Abbreviations used in this paper:* DC, dendritic cells; LC, Langerhans cells; tg, transgene.

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Methods

Mice. TGFβ1 null (−/−) and littermate control (+/+) mice were derived from matings of mice that were heterozygous for a TGFβ1 gene that had been disrupted by gene targeting (generated [16] and supplied by Ashok Kulkarni and Stephan Karlsson [NINDS, Bethesda, MD]). Since their derivation, heterozygous males have been backcrossed to C57BL/6 Ly-5.1$^+$ females on four occasions and the colony has been maintained by interbreeding. TGFβ1 null and littermate control mice used in these studies were reared in a pathogen-free facility and were used at 8–21 d of age.

Transgenic mice expressing a truncated dominant-negative TGFβ type II receptor in an epidermis-restricted pattern (under control of a truncated human loricrin promoter) were generated and character-

ized as described (15), bred at Baylor College of Medicine (Houston, TX) and transferred to the NIH for study. Female C57BL/6 (H-2^b, Ly 5.1), C57BL/6 (H-2^b, Ly 5.2), BALB/c mice (H-2^d), and BALB/c nu/nu mice (H-2^d) were obtained from Harlan Sprague-Dawley (Frederick, MD) and used at 8–16 wk of age. All animals were housed and used in experiments in accordance with institutional guidelines.

Flow cytometry and immunofluorescence microscopy. Hybridomas secreting mAb Y3-P (anti–I-A^b, mIgG2a), MK-D6 (anti–I-A^d, mIgG2a) and N418 (anti-CD11c, hamster IgG) were obtained from ATCC (American Type Culture Collection, Rockville, MD). mAb Y3-P, MK-D6, and N418 were purified from hybridoma supernatants by protein A affinity chromatography (Pierce Chemical Co., Rockford, IL). The anti–murine E–cadherin mAb DECMA-1 (rIgG1) was purchased from Sigma Chemical Co. (St. Louis, MO) as lyophilized ascites and purified using immobilized protein G (Pierce Chemical). mAb were modified, as indicated, with FITC (Sigma) or NHS-LC-biotin (Pierce Chemical) as described (17). Biotin-conjugated (Bio)-mAb 104 [anti–Ly-5.1, mIgG (18)] and Bio-A20 [anti–Ly-5.2, mIgG (18)] were provided by Al Singer (NCI, Bethesda, MD). The following mAb were purchased from PharMingen (San Diego, CA): FITC–anti-TCRαβ (hamster IgG), FITC–anti-TCRγδ (hamster IgG), Bio–anti-CD45 (rIgG2a) and relevant isotype controls. Phycoerythrin-streptavidin (PE-SA) was purchased from Tago, Inc. (Burlingame, CA).

For flow cytometry, cells were suspended in cold PBS containing 5% FBS and 0.02% NaN_3 and preincubated with saturating concentrations of 2.4G2 (anti-FcRγII [19] supplied as ascites fluid by Julie Titus [NCI, Bethesda, MD]) followed by serial incubations with FITC-mAb, Bio-mAb and PE-SA. Surface antigen expression was analyzed using a FACScan flow cytometer equipped with research software (Becton Dickinson, Mountain View, CA). Propidium iodide permeable (nonviable) cells were excluded by live gating. Epidermal sheets were stained for LC with saturating concentrations of FITC–anti-I-A diluted in PBS/FBS/NaN_3, washed and analyzed by epifluorescence microscopy.

Propagation of dendritic cells from bone marrow. DC were propagated from adult murine bone marrow as described by Inaba et al. (3). In brief, marrow was aspirated from the long bones of TGFβ1 null or littermate control mice, erythrocytes were depleted by osmotic lysis (ACK Lysing Buffer; Biofluids, Rockville, MD), and the remaining cells were cultured in complete media (RPMI 1640 containing 10% FBS, 10 mM Hepes [all from Biofluids], 1 mM glutamine [GIBCO BRL, Gaithersburg, MD], 10 mM pyruvate [GIBCO BRL], 50 μM 2-ME [Sigma Chemical Co.] and 20 μg/ml gentamycin [GIBCO BRL]) supplemented with murine rGM-CSF (10 ng/ml; R & D Systems, Minneapolis, MN) for 7 d. One-half of the culture media was replaced every 48 h.

Primary allogeneic reactions. DC propagated from the bone marrow of TGFβ1 null and control mice were co-cultured in flat-bottomed 96 well plates with 2×10^5 nylon wool nonadherent, anti–I-A plus complement-treated T cells prepared from the skin-associated lymph nodes of female BALB/c mice (20) for 120 h at 37°C. [^{3}H]TdR (1 μCi/well) was added for the final 12 h of the culture period. DC were irradiated (15 Gy) before addition into mixed leukocyte reactions. Cell-associated radioactivity was determined by direct beta counting.

Assessment of Langerhans cell precursors via bone marrow transplantation. Marrow was aspirated from the long bones of 8–21-d old TGFβ1 null and littermate control (H-2^b, Ly-5.1) mice as well as 6–8-wk old C57BL/6 Ly-5.2 mice. Marrow from multiple donors of each genotype was pooled and depleted of T cells via 2 cycles of anti–T cell (anti–Thy-1.2 [HO-13-4], anti–Ly-1.2 [C3PO] and anti–Ly-2.2 [83-12-S]) and guinea pig complement treatment as previously described (21). Each lethally irradiated (10 Gy) C57BL/6 Ly-5.2 female recipient received 5×10^6 T cell-depleted bone marrow cells by tail vein injection. Animals were assessed for chimerism and LC repopulation using Ly-5 alloantigen-specific mAb, appropriate lineage-specific reagents and multicolor flow cytometry. Weight gain was regularly determined and tissues of recipients were studied at necropsy.

Preparation of epidermal cell suspensions and epidermal sheets. Epidermal cell suspensions were prepared from trunk skin by limited trypsinization (0.25% trypsin [US Biochemicals, Cleveland, OH] in HBSS without calcium or magnesium at 4°C for 18 h) and dissociation of epidermal sheets by gentle pipeting in HBSS/ 0.05% DNase/30% FBS. Cell clumps were removed by passing the cell suspension through 50-μm nylon mesh. For analysis of LC in situ, epidermal sheets were prepared from engrafted trunk skin (see below) by incubation in 0.5 M ammonium thiocyanate (37°C for 20 min), fixed in acetone (30 min at −20°C) and rehydrated in PBS (22).

Assessment of cytokine mRNAs in skin by RNase protection. Subcutaneous fat was removed from the trunk skin of TGFβ1 null and control mice, and skin was snap frozen in liquid nitrogen. After ultrasonic homogenization (Polytron; Brinkmann Instruments, Westbury, NY), RNA was prepared using a modification of the guanidinium isothiocyanate method (CLONsep Total RNA Isolation Kit; Clontech, Palo Alto, CA). Cytokine mRNAs expressed in TGFβ1 and null skin were identified using an RNAse protection assay (23). Multi-probe templates specific for various murine cytokines were purchased from Pharmingen and radiolabeled probes were generated using T7 polymerase (Promega, Madison, WI) and [^{32}P]dUTP (NEN, Boston, MA). After hybridization with sample or yeast control RNA (∼ 0.25 μCi probe mixture with 5 μg RNA for 18 h at 45°C), protected fragments resistant to digestion with RNAse A and T1 (Pharmingen) were resolved in denaturing 8% polyacrylamide gels and detected by autoradiography.

Full-thickness skin grafts. BALB/c nu/nu mice were anesthetized with a mixture of ketamine (Fort Dodge Laboratories, Fort Dodge, IA) and xylazine (Mobay Corp., Shawnee, KS) and graft beds were prepared by the removal of trunk skin to the level of the panniculosus carnosus (24). Full-thickness skin grafts were obtained from the trunk skin of TGFβ1 null mice or wildtype mice, placed in the prepared beds, and secured with bandages for 10 d. Recipients were killed 12 wk after surgery and epidermal sheets from skin grafts and nongrafted skin were analyzed for the presence or absence of LC (see above).

Results

Propagation of dendritic cells from the bone marrow of TGFβ1 null mice. The LC abnormality identified in TGFβ1 null mice could reflect a deficiency of bone marrow-derived progenitors, or alternatively could result from the inability of LC (or their immediate precursors) to localize in epidermis. To address the former possibility we carried out several types of experiments. Initially, we attempted to propagate DC from the bone marrow from −/− mice using methodology previously described by Inaba and coworkers (3). Marrow was aspirated from the long bones of −/− mice (and +/+ littermate controls) and cultured in FCS-containing complete media supplemented with recombinant muGM-CSF (10 ng/ml). After 7 d, cells were recovered, enumerated and stained for the simultaneous expression of the DC marker CD11c and either I-A Ag or E-cadherin.

Multicolor flow cytometry demonstrated that −/− and +/+ bone marrow gave rise to populations of cells exhibiting similar forward and side scatter profiles (data not shown) and similar patterns of CD11c and I-A Ag expression (Fig. 1). The number of $CD11c^+$ I-A^+ cells (DC) recovered from cultures initiated with −/− and +/+ bone marrow was also similar ($5.5\pm2.2 \times 10^5$ and $6.4\pm3.4 \times 10^5$ DC per 10^6 bone marrow cells from −/− and +/+ mice respectively; $n = 4$ experiments). In addition, DC from TGFβ1 null marrow stimulated the proliferation of unprimed allogeneic T cells as well (or perhaps better) than DC from +/+ marrow (Fig. 2). Although the

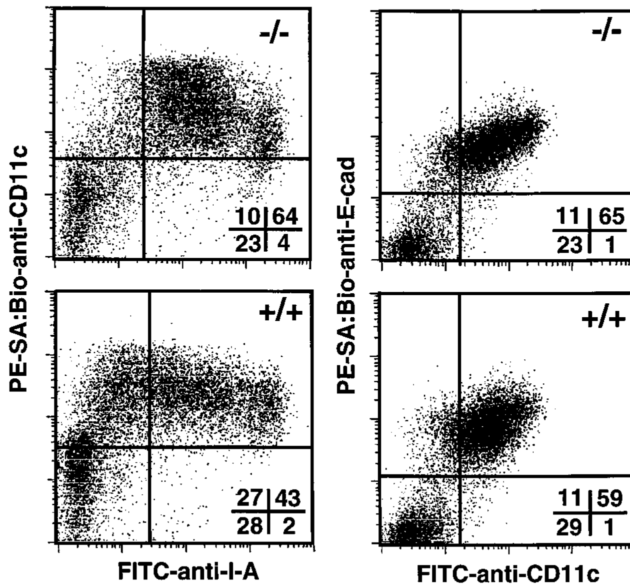

Figure 1. Propagation of dendritic cells from TGFβ1 null bone marrow. Bone marrow cells were harvested from the long bones of −/− and littermate control mice and erythrocytes were removed by osmotic lysis. Remaining leukocytes were incubated in complete media supplemented with recombinant muGM-CSF (10 ng/ml) for 7 d, recovered, and examined for expression of CD11c and I-A (or E-cadherin) using multicolor flow cytometry. Markers were set such that > 95% of all cells stained with isotype control mAb were contained within the left lower quadrant. Representative data from one of four experiments are depicted (n = 4).

Table I. Epidermal Langerhans Cells in Recipients of TGFβ1 Null Bone Marrow

Experiment	Duration	Donor genotype	Recipients	Donor I-A$^+$ (%)
I	8 wk	−/− (Ly-5.1)	n = 4	0.4±0.2
		+/+ (Ly-5.1)	n = 5	5.0±2.3
		+/+ (Ly-5.2)	n = 5	3.3±0.4
II	16 wk	−/− (Ly-5.1)	n = 4	3.9±2.1
		+/+ (Ly-5.1)	n = 5	3.3±0.9
		+/+ (Ly-5.2)	n = 5	3.8±1.0
III	16 wk	−/− (Ly-5.1)	n = 3	4.2±1.7
		+/+ (Ly-5.1)	n = 5	2.3±0.6
		+/+ (Ly-5.2)	n = 5	3.3±0.5
IV	8 wk	−/− (Ly-5.1)	n = 5	2.4±1.0
		+/+ (Ly-5.1)	n = 5	4.5±1.2
		+/+ (Ly-5.2)	n = 5	4.1±0.5
	16 wk	−/− (Ly-5.1)	n = 4	1.6±0.3
		+/+ (Ly-5.1)	n = 5	2.6±0.8
		+/+ (Ly-5.2)	n = 5	4.1±0.7

Lethally (10 Gy)-irradiated C57BL/6 Ly-5.2 female mice were engrafted with pooled T cell-depleted bone marrow cells from TGFβ1 null and control mice (5×10^6 cells per recipient). Donor-derived (Ly-5.1$^+$) LC (I-A$^+$ cells) were assessed in epidermal cell suspensions obtained from individual recipients by multicolor flow cytometry. Data represent percentages of viable cells in suspensions bearing the indicated alloantigens and are expressed as the mean±SEM of n determinations.

relationship of DC expanded from bone marrow in vitro to LC in situ is unclear, it is of interest that essentially all CD11c$^+$ cells in short-term cultures originated from −/− and +/+ bone marrow expressed E-cadherin (Fig. 1), an adhesion molecule that distinguishes LC (and perhaps their derivatives) from other lymphoid DC in vivo (25).

Langerhans cell precursors in the bone marrow of TGFβ1 null mice. To directly determine if the bone marrow of TGFβ1 null mice contained LC progenitors, we assessed the ability of bone marrow cells from −/− mice to give rise to LC in normal animals. T cell-depleted bone marrow cells from −/− or +/+ mice were adoptively transferred to lethally irradiated Ly-5 disparate recipients, mice were killed at 8 or 16 wk after transfer, and epidermal cell suspensions from trunk skin were examined for donor-derived LC (Ly-5.1$^+$, I-A$^+$ cells). Chimerism in recipients at the time of experiment termination ranged from 77 to 98%, depending on the experiment and the lineage studied (data not shown).

In an initial experiment harvested at 8 wk, bone marrow from −/− mice did not reconstitute LC as well as marrow from controls (Table I). However, in a second experiment terminated at 8 wk (Experiment IV) and three additional experiments assayed at 16 wk, bone marrow from −/− and +/+ mice generated similar numbers of epidermal LC. These results suggest that the LC deficiency that is evident in the epidermis of TGFβ1 null mice does not reflect an absolute deficiency in LC precursors in these mice.

Inflammation and proinflammatory cytokine synthesis in the skin of TGFβ1 null mice. TGFβ1 null (−/−) mice are indistinguishable from wildtype [(+/+) and (+/−)] littermates at birth and for the first 7–10 d of life (16, 26). Thereafter, −/− mice develop a progressive multiorgan inflammatory syndrome that invariably eventuates in wasting and death at 3–4 wk of age. The skin of −/− mice is grossly normal and cutaneous inflammation is not a prominent feature of the phenotype (data not shown). Although abrogation of the multiorgan inflammatory syndrome with rapamycin did not normalize LC numbers in −/− mice (14), we were concerned that failure of LC to localize in TGFβ1 null epidermis might reflect disordered cytokine synthesis within −/− epidermis. Specifically, we hypothesized that local overproduction of keratinocyte-derived TNFα or IL-1, two cytokines known to mobilize LC from skin, might cause the LC abnormality observed in TGFβ1 −/− mice.

We profiled 19 murine cytokine mRNAs, as well as IL-1 re-

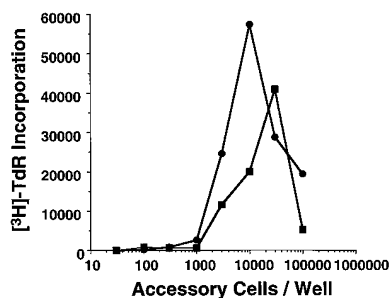

Figure 2. Allostimulatory activity of TGFβ1 null bone marrow-derived dendritic cells. DC were expanded from the bone marrow of −/− and +/+ mice in vitro in GM-CSF-supplemented media, irradiated (15 Gy), and their ability to stimulate the proliferation of unprimed BALB/c lymph node T cells was assessed. (*Closed circles*) DC from TGFβ1 null bone marrow; (*closed squares*) DC from control animals (n = 3).

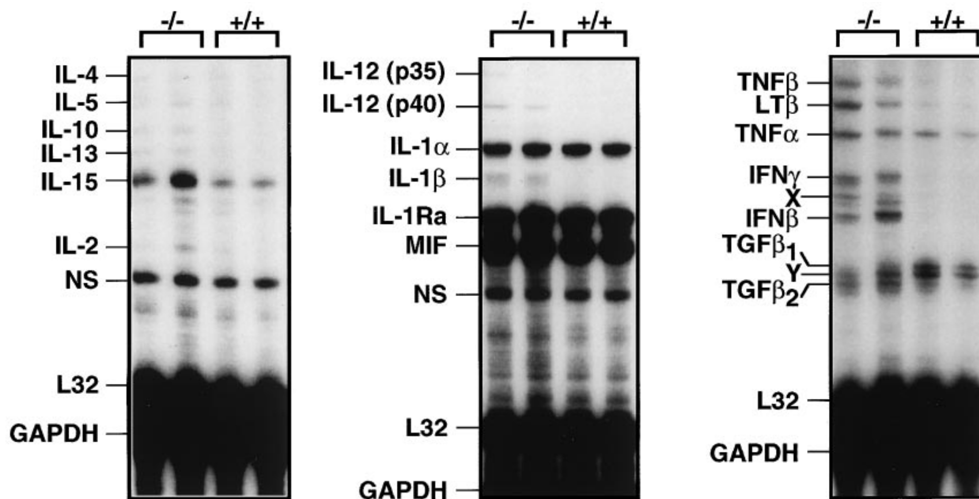

Figure 3. Cytokine mRNAs in TGFβ1 null and control skin. Total RNA was prepared from the trunk skin of 12-d old TGFβ1 null mice and littermate controls and mRNAs encoding various cytokines were assayed by RNase protection as described in Methods. Each lane represents the results obtained with RNA from a single animal.

ceptor antagonist mRNA, in 12-d old TGFβ1 null and littermate control skin using commercially available templates and a sensitive RNAse protection assay. Several cytokine mRNAs were overexpressed in TGFβ1 null skin. The most prominent changes were in TNFβ, LTβ, IFNγ, and IFNβ mRNAs (Fig. 3); smaller increases in IL-1β, IL-2, IL-4, IL-5, IL-10, IL-12 (p40), and IL-13 mRNA were also observed in autoradiographs exposed for longer time periods. Although TGFβ1 mRNA was not detected in the skin of −/− mice, TGFβ2 mRNA was perhaps somewhat more abundant. In addition, several unidentified protected species of unknown significance were detected in TGFβ1 −/− (bands X and Y) and control (band Y) epidermal RNA (Fig. 3). Although IL-1α, TNFα, and IL-1RA mRNAs were prominently represented in littermate control skin, their levels of expression were not higher in RNA from TGFβ1 null animals. Thus, it does not appear that proinflammatory cytokines that might mobilize LC, or that might prevent them from localizing in epidermis, are dramatically overproduced in TGFβ1 null skin.

Localization of Langerhans cells in TGFβ1 null skin. To determine if normal LC could localize in TGFβ1 null epidermis, BALB/c nu/nu mice were engrafted with full thickness trunk skin from −/− mice and controls. LC do not persist in grafts of full thickness murine skin; over time skin grafts are repopulated with recipient-derived LC. 12 wk after surgery, TGFβ1 null and littermate control skin grafts were removed and examined for the presence of BALB/c (I-A^{d+}) LC. Although BALB/c LC were observed in vertical sections of engrafted skin from −/− as well as +/+ skin using immunofluorescence microscopy (data not shown), results were more dramatic in epidermal sheet preparations. The morphology and density of I-A^{d+} LC in grafts from −/− skin, littermate control skin and nongrafted BALB/c nu/nu skin were indistinguishable (see Fig. 4).

Langerhans cells in epidermis-restricted dominant-negative TGFβ type II receptor transgenic mice. Previous studies demonstrated that TGFβ1 null skin cell suspension grafts contain immunoreactive TGFβ1 after engraftment onto animals that are competent to produce TGFβ1 and exhibit attenuated keratinocyte proliferation (27). Thus, it is possible that the TGFβ1 content of TGFβ1 null skin might normalize after engraftment onto BALB/c nu/nu mice (see above). To address the issue of the suitability of TGFβ1 −/− epidermis for LC localization in another way, we characterized epidermal leukocytes in the skin of mice that expressed a dominant-negative TGFβ type II receptor under control of a truncated loricrin promoter (15). The transgene in these animals is expressed in suprabasal as well as basal keratinocytes beginning on gestational d16, resulting in a transiently thickened hyperproliferative epidermis. Keratinocytes derived from these transgenic mice are also resistant to the growth inhibitory effects of TGFβ1 in vitro. Thus, although keratinocytes (and other cells) in dominant-negative TGFβ type II receptor transgenic mice produce TGFβ1, the keratinocyte hyperproliferative component of the TGFβ1 null phenotype is reproduced in these animals. Whether or not the cutaneous microenvironments in TGFβ1 −/− and dominant-negative TGFβ type II receptor transgenic mice are otherwise similar is unknown (see below).

Epidermal leukocytes in progeny that resulted from the matings of heterozygous dominant-negative TGFβ type II receptor transgenic parents (three litters) were characterized at a time when the hyperproliferative skin phenotype was evident (15). Homozygous transgenic animals died shortly after birth (15), and were not studied. Similar numbers of LC and dendritic epidermal T cells were detected in single cell suspensions of epidermal cells derived from the trunk skin of wild type and transgenic animals using analytical flow cytometry (see Table II). These results, in conjunction with those from the skin transplant studies, suggest that the epidermal microenvironment in TGFβ1 −/− mice is permissive for LC localization.

Discussion

Results of a previous study indicated that LC, the epidermal representatives of the DC lineage, are absent from the skin of TGFβ1 null mice (14). Although the LC defect could be dissociated from the inflammatory syndrome that is prominent in −/− mice, the nature of the requirement for TGFβ1 was not defined. To begin to address this issue, we undertook a series of experiments designed to determine if TGFβ1 exerted its major effect on LC (or LC progenitors) or on the cutaneous microenvironment. $CD11c^+$ MHC class II^{hi} E-cadherin$^+$ DC

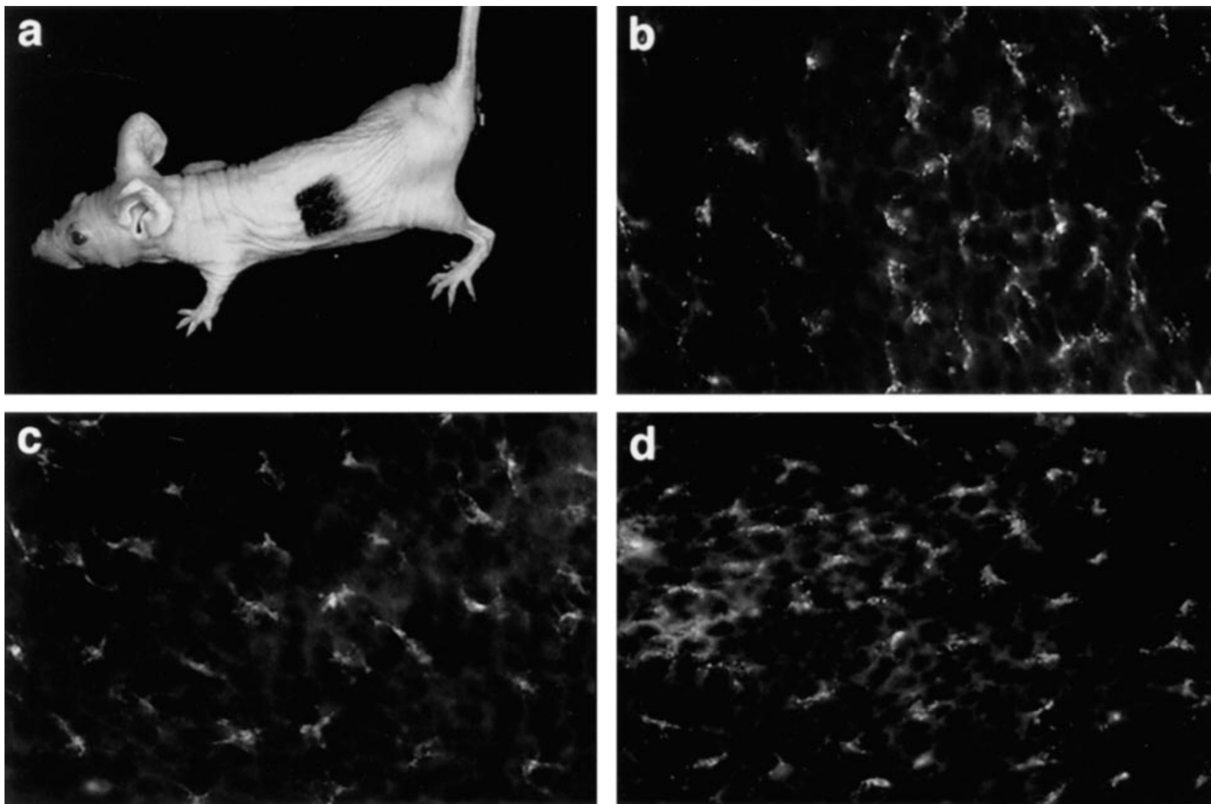

Figure 4. Localization of normal Langerhans cells in TGFβ1 null epidermis. BALB/c nu/nu mice were engrafted with full thickness abdominal skin from −/− and +/+ mice. 12 wk later grafts were harvested and recipient-derived LC were identified in ammonium thiocyanate-separated epidermal sheets using immunofluorescence microscopy. (*a*) BALB/c nu/nu mouse engrafted with TGFβ1 null skin. (*b*) I-A^d-bearing LC in epidermis of −/− graft (n = 4). (*c*) I-A^d-bearing LC in epidermis of +/+ graft (n = 4). (*d*) I-A^d-bearing LC in recipient (ungrafted) BALB/c nu/nu epidermis.

with potent allostimulatory activity were readily propagated from TGFβ1 null as well as littermate control bone marrow in serum-containing media. In addition, similar numbers of LC were identified in the epidermis of lethally irradiated Ly-5 disparate mice after reconstitution with T cell-depleted TGFβ1 null and control marrow. Thus, the absence of LC from the epidermis of −/− mice does not reflect an absolute deficiency of bone marrow precursors.

Table II. Epidermal Leukocytes in Epidermis-restricted Dominant-negative TGFβ1 Type II Receptor Transgenic Mice

Age	Genotype	I-A^+	TCRγδ^+	$CD45^+$
d		%	%	%
5	+/tg	5.0	0.6	6.6
	+/tg	4.7	0.5	7.0
	+/tg	7.3	0.4	8.3
6	+/+	6.1	2.9	9.5
	+/tg	6.5	1.1	9.1
	+/tg	5.8	0.8	6.7
7	+/+	7.2	1.2	9.1
	+/tg	3.8	0.7	4.8
	+/tg	7.8	1.6	10.4

Epidermal cell suspensions were prepared from the trunk skin of mice that resulted from the mating of mice that were heterozygous for a dominant-negative TGFβ type II receptor transgene expressed in epidermis under the control of a truncated loricrin promoter. LC (I-A^+ cells) and dendritic epidermal T cells (TCRγδ^+ cells) were identified and quantitated in cell suspensions using analytical multicolor flow cytometry.

To determine if the skin of −/− mice was receptive to LC localization, we carried out several kinds of experiments. First, we characterized cytokine mRNA levels within the cutaneous microenvironment. TNFβ, LTβ, IFNγ, and IFNβ mRNAs were clearly overrepresented in TGFβ1 null skin, and TGFβ2 mRNA levels may also be somewhat increased. These latter data are in contrast with that from previous studies in which compensatory increases in TGFβ2 and TGFβ3 production were not detected in TGFβ1 −/− mice by Northern analysis and/or immunohistochemistry (16, 28). Importantly, mRNAs encoding cytokines known to activate and mobilize LC from epidermis (IL-1 and TNFα) (11, 12, 29, 30) were not markedly elevated in −/− skin. Second, to address the issue more directly, we assessed the ability of normal LC to localize in transplanted TGFβ1 skin. Similar numbers of bone marrow-derived LC were identified in −/− and control skin after engraftment onto BALB/c nu/nu mice. In addition, LC density in the grafts was not obviously different from that in nongrafted BALB/c nu/nu skin.

We also characterized the epidermal leukocytes in transgenic mice expressing a dominant-negative TGFβ type II receptor in an epidermis-restricted pattern under control of a truncated loricrin promoter (15). The epidermis of these

animals had numbers of LC and DETC that were indistinguishable from littermate controls. Keratinocytes from epidermis-restricted dominant-negative TGFβ type II receptor transgenic mice are unresponsive to the growth inhibitory effects of TGFβ1 in vivo and in vitro, and the keratinocyte hyperproliferative component of the TGFβ1 null skin phenotype is recapitulated in transgenic skin (15). Whether or not other features of the TGFβ1 −/− skin phenotype are present in the transgenic mice is unknown. Currently there is some disagreement about the requirement for type I and type II TGFβ receptors for TGFβ1 signaling. Chen and Feng and coworkers have suggested that TGFβ1-induced growth inhibition and gene expression can be uncoupled in certain situations, and that TGFβ1 can stimulate gene expression (but not growth inhibition) in cells that lack functional type II receptors (31, 32). Using the same strategy and similar constructs, Wieser et al. demonstrated a requirement for both receptors for both types of responses (33). An explanation for this discrepancy is not immediately apparent, and the controversy has not yet been resolved. Thus, although we have no evidence that keratinocytes in TGFβ1 null mice and the transgenic mice that we studied differ significantly (apart from their ability to produce TGFβ1), it is possible that they are quite dissimilar.

Nonetheless, in aggregate, our results suggest that the LC deficiency in TGFβ1 null mice reflects a requirement of LC (or LC precursors) for TGFβ1, and that the cytokine does not exert its effect by modulating the cutaneous microenvironment. This conclusion is consistent with the results of in vitro experiments that have recently been carried out by several groups. Strobl and coworkers have reported that the outgrowth of CD1a$^+$ Birbeck granule-containing cells (LC) from human CD34$^+$ cells in a defined serum-free system was absolutely dependent on the addition of TGFβ1 (34). More recently, these investigators demonstrated that, in this system, TGFβ1 protects LC progenitors from apoptosis (35). In the present study, we report that dendritic cells can be expanded from TGFβ1 null bone marrow in serum (and TGFβ1)-containing media. Unfortunately, serum-free culture conditions that allow growth of murine LC have not been developed so we cannot as yet determine if TGFβ1 is required for survival and proliferation of mouse as well as human cells.

Alternatively (or in addition), TGFβ1 may influence LC homing, perhaps by regulating the expression of important adhesion molecules. Strunk et al. recently determined that human LC progenitors are contained within the subset of CD34$^+$ peripheral blood cells that are also CLA$^+$ (36). Although CD1a$^+$ HLA-DR^{bright} DC could be expanded from both CLA$^+$ and CLA$^-$ cells, only CD34$^+$ CLA$^+$ cells gave rise to cells containing Birbeck granules (a hallmark of LC). Picker and coworkers have previously shown that expression of CLA, a well known ligand for E-selectin and a marker for skin homing T cells, is positively regulated in human lymphocytes by TGFβ1 (37). Whether or not CLA expression is TGFβ1-dependent in human LC is unknown, just as it is unknown if CLA is involved in LC localization. However, it is possible that the LC deficiency in TGFβ1 null mice occurs because LC, or their progenitors, do not express an adhesion molecule (such as the murine homologue of CLA) that is essential for homing to skin. This hypothesis can be tested when anti-murine E-selectin ligand reagents become available.

TGFβ1 could also regulate LC localization by modulating differentiation in other ways. Experiments with relB null mice, another mouse whose phenotype is comprised in part by a multiorgan inflammatory syndrome, suggest that this member of the NFκB/rel family of transcription factors is an important regulator of DC (38, 39). RelB null mice have normal numbers of LC, but lack lymphoid DC. Because DC selectively express high levels of relB (40), it is reasonable to propose that relB is involved in the transformation of LC into DC. Arsura et al. recently reported that TGFβ1 induces accumulation of IκBα, an endogenous inhibitor of NFκB/rel transcription factors (including relB) in murine B cells (41). If the same is true in cells of the DC lineage, it is possible that TGFβ1 may maintain LC in the differentiation state that is characteristic of nonlymphoid DC by inhibiting relB. Preliminary experiments indicate that LC are present in normal numbers in the epidermis of 5–6-d old IκBα null mice, but that they exhibit a phenotype that resembles that of LC which have been activated by contact allergens and certain proinflammatory cytokines (Klement, J.P., and M.C. Udey, unpublished observations). These results do not implicate IκBα as an important mediator of TGFβ1 effects on LC, but they do add support to the concept that NFκB/rel transcription factors are important regulators of DC.

TGFβ1 is a pleiotropic cytokine, and relevant biochemical pathways through which TGFβ1 exerts its effects on LC remain to be determined. Recently, we have identified conditions that allow expansion of LC-like cells in primary cultures of murine fetal skin cells (Jakob, T., and M. C. Udey, manuscript submitted for publication). Because these cells exhibit phenotypes like LC in situ and lymphoid DC at different stages of development, this system will facilitate studies of mechanisms that promote the transformation of nonlymphoid DC into lymphoid DC and should allow us to gain additional insights into the role that TGFβ1 plays in LC biology.

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