Human Skin/SCID Mouse Chimeras as an *In Vivo* Model for Human Cutaneous Mast Cell Hyperplasia

Melpo Christofidou-Solomidou,* B. Jack Longley,† Diana Whitaker-Menezes,‡ Steven M. Albelda,* and George F. Murphy‡

*Departments of Medicine and Dermatology, University of Pennsylvania Medical Center, Philadelphia, Pennsylvania, U.S.A.; †Department of Dermatology, Yale University School of Medicine, New Haven, Connecticut, U.S.A.; and ‡Department of Pathology, Anatomy and Cell Biology, Thomas Jefferson Medical College, Philadelphia, Pennsylvania, U.S.A.

Human skin xenografted to mice with severe combined immunodeficiency syndrome (SCID) was evaluated to determine the integrity and fate of human dermal mast cells. There was an approximately 3-fold increase in number of dermal mast cells by 3 mo after engraftment ($p < 0.05$). These cells were responsive to conventional mast cell secretagogues and were confirmed to be of human origin by ultrastructural characterization of granule substructure and by reactivity for the human mast cell proteinase, chymase. CD1a+ Langerhans cells, also bone marrow–derived cells, failed to show evidence of concomitant hyperplasia, and increased mast cell number was not associated with alterations in number of dermal vascular profiles identified immunohistochemically for human CD31. RT-PCR analysis demonstrated human but not murine stem cell factor (SCF; also termed mast cell growth factor, c-*kit* ligand) mRNA in xenografts. Epidermal reactivity for stem cell factor protein shifted from a cytoplasmic pattern to an intercellular pattern by 3 mo after engraftment, suggesting a secretory phenotype, as previously documented for human cutaneous mastocytosis. The majority (>90%) of mast cells demonstrated membrane reactivity for human SCF at the time points of peak hyperplasia. These data establish SCID mouse recipients of human skin xenografts as a potential *in vivo* model for cutaneous mast cell hyperplasia. ***Key words: mastocytosis/stem cell factor/xenografts. J Invest Dermatol 109:102–107, 1997***

Cutaneous mastocytosis is characterized by (i) diagnostically significant mast cell (MC) hyperplasia within the dermis (Murphy, 1995); (ii) localization of MCs in intervenular interstitium in addition to more normal angiocentric loci (Walsh *et al*, 1991); and (iii) phenotypic shifts in epidermal expression of MC growth factor or stem cell factor (SCF) (Longley *et al*, 1993; Weiss *et al*, 1995). The mechanism(s) responsible for these events in mastocytosis and in MC hyperplasias unrelated to specific mastocytosis syndromes are poorly understood, in part because *in vivo* models that recapitulate human disease have not as yet been developed. For example, it remains unclear as to whether mastocytosis involves a defect intrinsic to MCs or rather to regulatory molecules in their microenvironment that govern their differentiation and proliferation (Longley, 1994). Moreover, some workers have hypothesized that mastocytosis is a form of cellular hyperplasia (Weidner and Austen, 1990), whereas others regard some forms of this disorder to show characteristics of clonality (Mirowski *et al*, 1990; Longley *et al*, 1996).

A recent model with promise for *in vivo* study of human cutaneous inflammation and neoplasia involves human skin xenografts (HSX) transplanted to mice with severe combined immune deficiency syndrome (SCID) (Bosma *et al*, 1983). Skin so transplanted maintains human function and phenotype and may be manipulated experimentally over prolonged intervals of time. Using the SCID/HSX model, we and others have documented acantholytic blister formation after systemic reconstitution with pemphigus lymphocytes (Juhasz *et al*, 1993a), growth and invasion of human melanoma cells (Juhasz *et al*, 1993b), regulation of extracellular matrix molecules and integrin adhesion receptors during wound healing (Juhasz *et al*,1993c) and reproduction of human lichenoid dermatitis (Christofidou-Solomidou *et al*, 1997) and delayed hypersensitivity reaction (Petzelbauer *et al*, 1996).

In the course of developing further the SCID/HSX model for the study of the inflammatory cascade in xenografted human skin (Yan *et al*, 1993, 1994; Christofidou-Solomidou *et al*, 1996), we observed increases in numbers of dermal MCs so that the xenografted skin eventually developed a striking similarity to lesions of human cutaneous mastocytosis.[1] The purpose of the present study was to characterize the xenografts in terms of (i) expression of the human MC phenotype; (ii) the kinetics of lesion formation; (iii) potential associated changes in related skin cell populations; and (iv) expression of SCF required for MC differentiation and proliferation.

Our findings indicate that by 3 mo after xenografting of human skin to SCID mice, dramatic increases in dermal MC numbers are observed. These cells are of human phenotype and maintain

Manuscript received December 6, 1996; revised March 18, 1997; accepted for publication March 21, 1997.

Reprint requests to: Dr. George F. Murphy, Jefferson Alumni Hall, Suite 545, 1020 Locust Street, Philadelphia, PA 19107-6799.

Abbreviations: HSX: human skin xenograft; MC: mast cell; SCID: severe combined immunodeficiency syndrome; SCF: stem cell factor.

[1] Christofidou-Solomidou M, Albelda SM, Murphy GF: A novel *in vivo* model for mastocytosis: hyperplasia of human dermal mast cells in skin xenografted to immunodeficient mice. *J Invest Dermatol* 102:611, 1994 (abstr).

features of cutaneous MC hyperplasia for up to 8 mo after engraftment, are responsive to conventional MC secretagogues, and are associated with expression of human SCF, which displays a secretory pattern identical to that previously documented in naturally occurring human mastocytosis (Longley *et al*, 1993). This model therefore holds promise for further exploration of the causes and potential treatments for disorders characterized by abnormally increased numbers of cutaneous MCs.

MATERIALS AND METHODS

Skin Transplantation The protocols for skin transplantation have been described previously in detail (Juhasz *et al*, 1993a; Yan *et al*, 1993; Christofidou-Solomidou *et al*, 1996). Briefly, SCID mice were obtained from a colony maintained at the Wistar Institute Animal Facility in a pathogen-free environment. At 4–6 wk of age, the plasma of each mouse was tested for IgM production, and only fully immunodeficient mice were used for tissue transplantation. Full thickness neonatal foreskins from elective circumcisions were cut into two halves, trimmed to a diameter of 1–1.5 cm, and grafted onto the flanks of the SCID mice in wound beds prepared by removing size-matched circular pieces of mouse skin down to the fascia. The human skin grafts were held in place by nonabsorbable suture material and covered with dressing clipped to the dorsal skin of the animal by means of a surgical staple. The dressing was removed after 1–2 wk and grafts were allowed to heal further.

Induction of MC Degranulation Several known MC secretagogues were tested for their ability to induce human MC degranulation after intradermal injection (see below). The substances used were: (i) morphine sulfate (Wyeth, Philadelphia, PA) used at 2 mM; (ii) substance P (5 mM) (Sigma Chemical Inc., St. Louis, MO); and (iii) compound 48/80 (Sigma) used at 0.5 mg per ml. Concentrations were derived from previous studies (Christofidou-Solomidou *et al*, 1996).

Administration of the secretagogues was performed as follows: A skin graft on one side of each mouse was injected intradermally with 50 μl of endotoxin-free saline containing 2 mg bovine serum albumin per ml (Sigma) as a protein carrier plus colloidal carbon (Sigma) to mark the site of injection (control side). The skin graft on the contralateral side was injected with the secretagogue diluted in 50 μl of endotoxin-free saline containing 2 mg bovine serum albumin per ml with colloidal carbon (experimental side). All injections were administered centrally within grafts using 28 gauge needles to minimize trauma.

Tissue Preparation Mice were sacrificed during monthly intervals over a 3-mo period, and a fourth group designated as >3 mo consisted of animals sacrificed from 4–8 mo after transplantation. Human skin xenografts were dissected from the animals, and the explants were cut into smaller pieces, oriented, placed in OCT compound, and snap-frozen for immunohistochemical analysis or triaged and prepared for electron microscopy. A total of 32 xenografts were studied and a minimum of four animals were sacrificed for each of the four groups (1, 2, 3, and >3 mo). In addition, all groups were compared to baseline values for neonatal foreskin at the time of transplantation ($T = 0$; $n = 4$).

Cryomicrotomy and Immunohistochemistry Serial sections of 6 μm thickness were cut from frozen specimens, fixed in cold acetone, and stored at −70°C. Prior to staining, sections were blocked with 5% serum appropriate for the secondary antibody used and incubated with the primary antibody for 1 h at room temperature or overnight at 4°C. The bound antibody was detected by use of an avidin-biotin-peroxidase complex (Vector Labs, Burlingame, CA) procedure with 3-amino-9-ethyl carbazole as the chromagen.

Immunoperoxidase staining was performed as previously described (Christofidou-Solomidou *et al*, 1996). No counterstaining was used in order to increase sensitivity. The following monoclonal antibodies were used for immunostaining: (i) mouse anti-human platelet endothelial cell adhesion molecule-1 (CD31) (AMAC, Westbrook, ME); (ii) mouse anti-human SCF, (Genzyme, Cambridge, MA); (iii) mouse anti-human CD1a (Becton Dickinson, San Jose, CA); and (iv) mouse anti-HMB-45 (Biogenex, San Ramon, CA). The following polyclonal antibodies were used: (i) goat-anti-human SCF, (R&D Systems, Minneapolis, MN); (ii) goat-anti mouse SCF (R&D Systems); (iii) rabbit-anti S-100 (Biogenex, San Ramon, CA); and (iv) anti-human chymase raised in rabbit against human granule-specific serine protease chymase, donated by Dr. N. Schechter (Schechter *et al*, 1986). Each antibody was titrated to obtain optimal expression with minimal background staining. Controls consisted of omission of the primary antibodies and of substitution of primary antibody incubations with irrelevant controls i.e., mouse IgG_1 antibody raised against an irrelevant antigen (Keyhole Limpet Hemocyanin) (Becton Dickinson, San Jose, CA), normal rabbit IgG, and normal goat IgG (Jackson Immunoresearch, West Grove, PA).

Double Labeling Immunofluorescence Microscopy Sections were blocked and incubated with the monoclonal primary antibody to human SCF raised in mouse and polyclonal human chymase raised in rabbit, followed by fluorescently labeled secondary antibodies using Texas Red and fluorescein isothiocyanate as fluorochromes (Accurate Scientific, Westbury, NY). As a positive control, single labeling with each antibody was performed on serial sections, and double negative controls were accomplished in which non-immune rabbit IgG was used instead of the chymase polyclonal antibody, and mouse IgG_1 to an irrelevant antigen (Becton Dickinson) instead of the SCF mouse monoclonal antibody. The sections were viewed with an MRC-600 confocal fluorescent microscope (Bio-Rad Microscience, Hercules, CA).

Electron Microscopy Selected biopsies demonstrating hyperplasia were evaluated using transmission electron microscopy as described previously (Whitaker-Menezes *et al*, 1995). Briefly, explants were fixed in Karnovsky's fixative (Karnovsky, 1965), washed, dehydrated with a graded series of ethanol to propylene oxide, and embedded in epon. Ultrathin sections were cut on an LKB ultrotome III (LKB, Bromma, Sweden), contrasted with uranyl acetate followed by bismuth subnitrate, and viewed with a Hitachi H7000 electron microscope (Hitachi Instruments, Elmsford, NY).

Reverse Transcriptase-Polymerase Chain Reaction (PCR) Punch biopsies (6 mm) were taken *ex vivo* from either excised skin xenografts following variable times of engraftment on the host animals (SCID mice) or from skins prior to transplantation representing the baseline samples. The biopsies were taken from the center of the xenografts to avoid the murine-human skin junctional regions. The epidermis was separated from the dermis by overnight incubation in a dispase solution (Collaborative Biomedical Products, Bedford, MA) at 37°C (Matsumura *et al*, 1975). Total RNA was extracted from the epidermal and dermal explants as described by Chromszynski and Sacchi (1987). Complementary DNAs were transcribed from total RNA with avian myeloblastosis virus reverse transcriptase, using random hexamers as primers (2 picomol per ml, Pharmacia LKB, Piscataway, NJ) as described by Longley *et al* (1991). Oligonucleotide primers corresponding to specific sequences in human SCF cDNA and DNA amplification was performed as described (Longley *et al*, 1993; Weiss *et al*, 1995).

Gel electrophoresis and southern blotting of PCR products, probe labeling and hybridization, subcloning, and sequencing were done according to Longley *et al* (1993) and Weiss *et al* (1995).

Statistics MCs were enumerated as chymase-positive cells/mm^2 with the assistance of an ocular grid micrometer. Monthly interval data were grouped and analyzed. Differences among groups were analyzed using an analysis of variance ($p < 0.05$).

RESULTS

Human MCs Undergo Progressive Hyperplasia in Xenografts Neonatal foreskin prior to xenotransplantation contained 45 ± 5 (mean ± SEM) human chymase-positive MCs situated primarily about superficial dermal vessels **(Fig 1*A*)**. These cells contained numerous, tightly compacted cytoplasmic granules that stained strongly for human chymase (murine MCs adjacent to graft anastomosis were nonreactive). During the first and second months, MCs shifted from a normal perivascular to a more diffuse interstitial pattern of distribution **(Fig 1*B–D*)**. The number of MCs gradually increased until >3 mo after engraftment, when chymase-positive cells increased to a maximum of 161 ± 23 cells/mm^2 ($p < 0.05$). **Figure 2** summarizes the evolutionary changes in MC number at timepoints examined. The human phenotype of the hyperplastic MCs was confirmed further by conventional ultrastructure, which demonstrated complex cytoplasmic granules of characteristic human phenotype (Whitaker-Menezes *et al*, 1995) **(Fig 3)**. These granules contained subcompartments composed of electron-dense amorphous zones, crystalline lattice, and scroll-like matrices. In contrast, MCs in adjacent murine skin were characterized by typical diffusely homogeneous, electron-dense granules. Occasional cells exhibited prominent Golgi zones and small cytoplasmic progranules consistent with active granulopoiesis (Dvorak *et al*, 1986) **(Fig 3*A*)**. Evidence of human/murine MC chimerism was not observed within the human xenografts.

In order to determine whether hyperplasia involved bone mar-

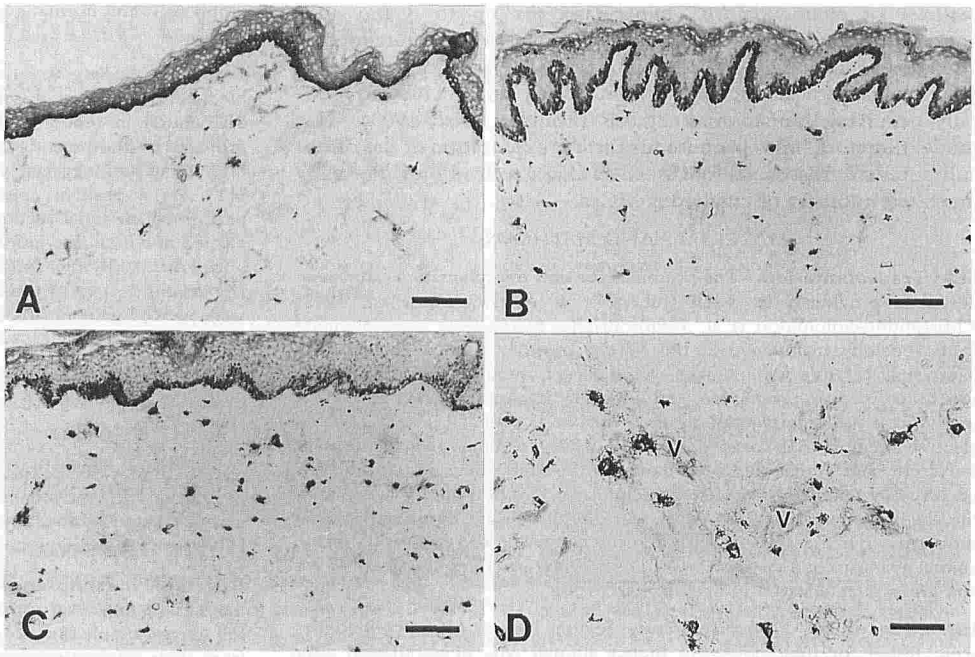

Figure 1. Immunohistochemical staining for human chymase-positive MCs in xenografts at varying timepoints after engraftment. *(A)* time 0; *(B)* 1 mo; *(C)* 3 mo; *(D)* 4 mo. *(D)* is higher magnification of granulated mast cells in 4 mo xenograft; (V) represents dermal vessels. *Scale bars: (A–C)* 200 μm; *(D)* 50 μm.

row–derived cells other than MCs, xenograft specimens were evaluated immunohistochemically for CD1a. Although Langerhans cells persisted in xenografts at all timepoints, significant alterations in cell number were not observed. The values over an observation period of 4 mo ranged from 11 to 15 ± 3 CD1a-positive cells per linear mm of epidermis. In order to exclude the possibility that MC hyperplasia was related to angiogenesis within grafts, representative samples were stained for human CD31 (platelet-endothelial cell adhesion molecule-1). Vessel profiles/mm^2 actually decreased over time from baseline (e.g., 67 ± 7 to 39 ± 12 vessels/mm^2 at 8 mo) in all groups of xenografts harvested after 3 mo from time of transplantation. Stains for S-100 protein failed to reveal increased numbers of epidermal melanocytes at timepoints of MC hyperplasia, and induction of HMB-45 on melanocytes was not observed (data not shown).

Hyperplastic Human MCs Are Normally Responsive to MC Secretagogues Direct intradermal injection of MC secretagogue compound 48/80, substance P, or morphine sulfate into xenografts at timepoints of peak MC hyperplasia **(Fig 4*A*)** resulted in rapid diminution of chymase staining by 30 min **(Fig 4*B*)**. Degranulation was evidenced by marked reduction in cytoplasmic granule number and staining intensity, granule enlargement, and apparent discharge of granules into the pericellular matrix. Transmission electron microscopy further confirmed the effects of secretagogues, showing loss of granule density and internal substructure, merging of adjacent granule membranes, coalescence of granule membranes with the MC plasma membrane, and extrusion of granule contents into the extracellular space **(Fig 4*D*)** (Caufield *et al*, 1990; Kaminer *et al*, 1991). Control injections of saline failed to produce evidence of degranulation.

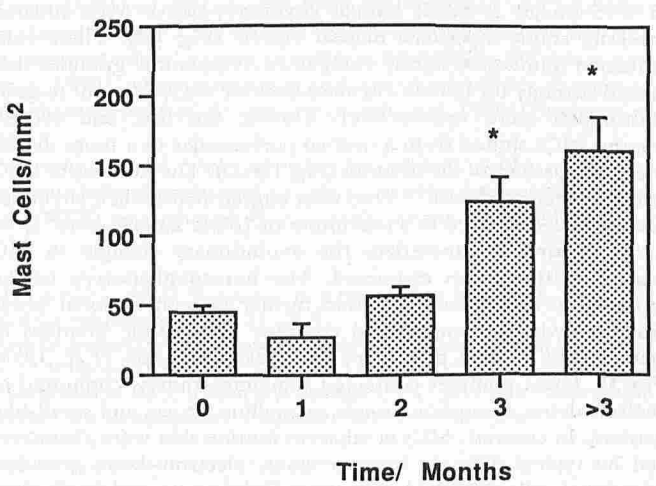

Figure 2. Graphic representation of numbers of human chymase-positive MCs/mm^2 after xenoengraftment. $p < 0.05$ for 3- and >3-mo groups *versus* untransplanted (T = 0), 1- and 2-mo groups. (*Error bars*, SEM).

Human SCF Synthesis Persists in Xenografts and SCF Immunoreactivity Shifts to a Membrane Pattern Reverse transcriptase-PCR analysis of xenografts selected at peak intervals of human MC hyperplasia demonstrated that human SCF mRNA is persistent in both epidermal and dermal explants of xenografts as 359- and 275-bp bands **(Fig 5*A*)**. Murine SCF mRNA, however, was not detected **(Fig 5*B*)** in either epidermal or dermal graft components, although it was readily detected in control murine skin (data not shown).

Immunohistochemical evaluation for SCF revealed a normal weak cytoplasmic pattern within keratinocytes at time 0 **(Fig 6*A*)**; 3 mo after engraftment **(Fig 6*B*)**, staining shifted to an intercellular pattern. Occasional dermal cells were also stained at this time. Confocal microscopy of preparations co-labeled for human chymase and SCF (**Fig 6*C,E*** and **6*D,F*,** respectively), demonstrated membrane-associated SCF coating chymase-positive dermal MCs at 3 mo after engraftment **(Fig 6*F*)** but not prior to this point **(Fig 6*D*)**. Antibodies to murine SCF failed to decorate cells expressing human chymase at any of the times examined (data not shown).

DISCUSSION

In this study, we demonstrate that human skin xenografted to immunodeficient mice undergoes progressive MC hyperplasia and that these cells express human granule proteinases and ultrastructural phenotype. Although these cells are increased in number and show an abnormal interstitial distribution pattern, they respond to conventional MC secretagogues. MC hyperplasia within xenografts appears to be relatively selective and is not associated with similar increases in Langerhans cells or angiogenesis. Human, but not murine, SCF mRNA is demonstrable within xenografts, and im-

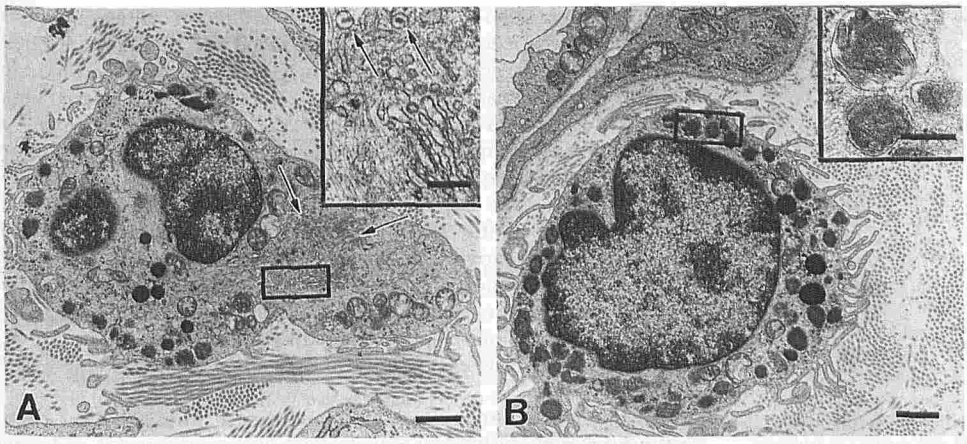

Figure 3. Ultrastructural examination of human MCs in 4-mo-old xenografts. *(A)* MC with *rectangular enclosure and inset* defining prominent Golgi zone (*rectangular enclosure*) and Golgi-associated progranule formation (→). *(B)* MC containing numerous mature granules with internal scrolls (*rectangular enclosure* and *inset*) *Scale bars: (A,B)* 1 μm; *(A inset)* 0.25 μm; *(B inset)* 0.5 μm.

munoreactive SCF protein is associated with keratinocyte and dermal MC membranes at times of the hyperplastic responses.

The mechanism of MC hyperplasia in human skin/SCID xenografts may involve (i) induction of MC differentiation from agranular precursors within the dermis; (ii) local replication of differentiated MCs; or (iii) inhibition of MC apoptosis. Evidence of Golgi-associated progranule formation with features similar to putative primitive MCs in human mastocytosis (Mirowski *et al*, 1990) and to developing MCs *in vitro* (Valent *et al*, 1989) was documented, suggesting that at least some of the observed increases in MC number resulted from granulopoiesis. Because xenografts are divorced from circulating human MC precursors, granulopoiesis would necessarily derive from resident dermal monocytic cells induced to undergo local differentiation or division. Mature MCs rarely divide, although mastocytosis cells have been documented to show metaphase and S-phase activity (Tsai *et al*, 1991). We did not note mitotic figures by routine microscopy or ultrastructure.

An increase in MC number remarkably similar to that shown in the SCID/HSX model occur in inflammatory dermatoses as well as in adult human mastocytosis. In the latter, this is associated with alteration of expression of SCF (Longley *et al*, 1993). These alterations consist of a shift in epidermal SCF distribution from a constitutive cytoplasmic pattern to an intercellular pattern consistent with secretion. In addition to promoting MC differentiation and replication, SCF is known to prevent MC apoptosis (Inemura *et al*, 1994; Mekori and Metcalfe, 1995). Therefore, the gradual increase in MC numbers within the xenografts could also be the result of increased MC survival. Tritiated thymidine incorporation studies or *in situ* labeling for endonuclease-mediated DNA fragments (Gilliam *et al*, 1996) could potentially clarify the mechanism leading to increased MC numbers in xenografts.

Although human SCF redistribution and secretion may partially account for the MC hyperplasia in the present study, melanocytic hyperplasia or HMB-45 induction, as may occur locally at SCF injection sites *in vivo* (Galli *et al*, 1993; Costa *et al*, 1996; Grichnick *et al*, 1995), was not observed. This could be the result of intrinsic differences between human skin *in vivo* and human skin xenografts, differences in SCF concentration threshold necessary for MC *versus* melanocyte stimulation, or induction of as yet undefined factors that selectively influence MC kinetics. The reason for human SCF redistribution in xenografts is unclear. It is possible that removal of

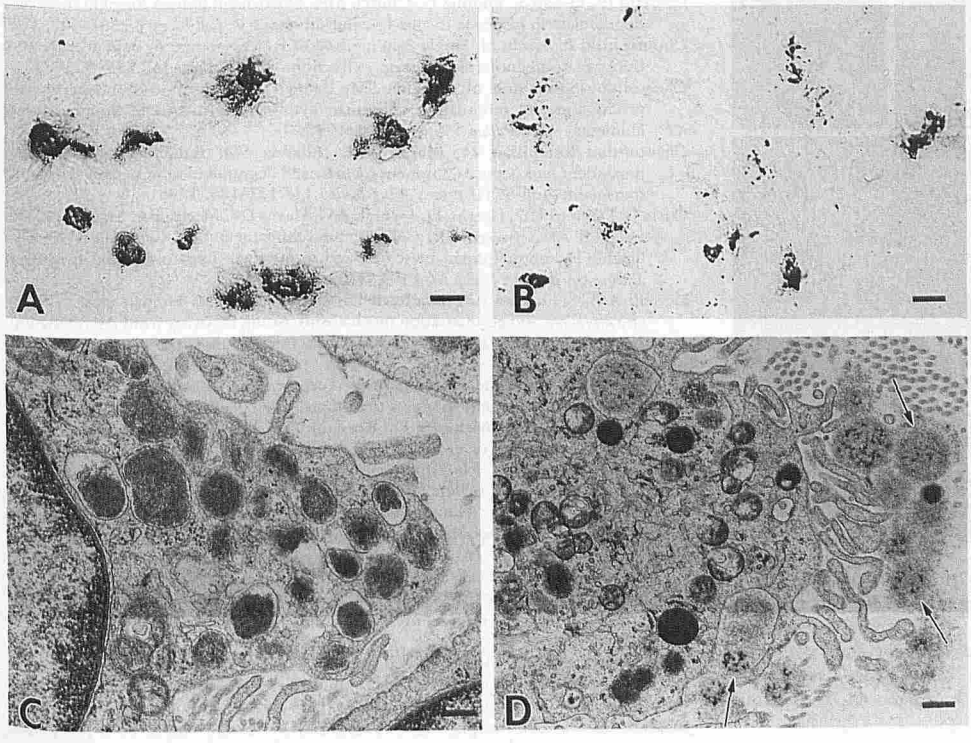

Figure 4. Response of 4-mo old xenograft MCs to injected secretagogue, compound 48/80. *(A)* Chymase immunohistochemistry, before *(A)* and after *(B)* stimulation. Ultrastructure before *(C)* and after *(D)* stimulation (→ in *(D)* indicate discharged granule contents). *Scale bars: (A&B)* 10 μm; *(C,D)* 0.5 μm.

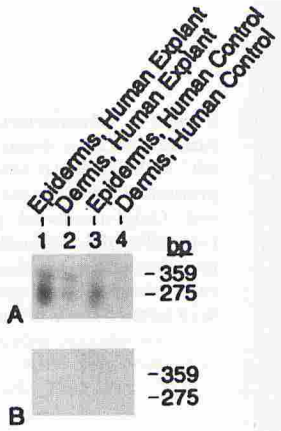

Figure 5. Reverse transcriptase-PCR detection of human *(A)* and murine *(B)* SCF mRNA in three epidermal and dermal explants from 3-mo-old xenografts.

xenografts from factors in the human circulation that potentially inhibit SCF could also account for this finding, and this possibility is being evaluated. Although contribution of murine SCF was excluded by PCR analysis and immunohistochemistry, it remains possible that altered human SCF expression could relate to exposure of human skin grafts to other factors derived from the nutrient murine microcirculation.

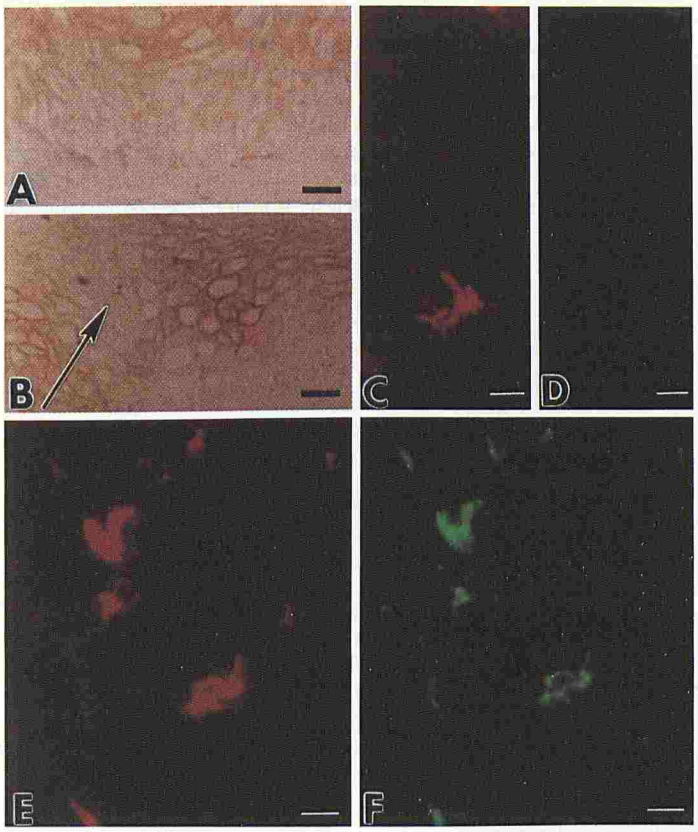

Figure 6. Immunohistochemical and confocal immunofluorescence evaluation of human SCF in xenografts at time 0 *(A,C,D)* and 3 mo *(B,E,F)* after transplantation. *(A)* and *(B)* represent epidermal immunohistochemical staining for SCF; *(C)* and *(E)* represent confocal immunofluorescence staining for MC chymase; *(D)* and *(F)* represent confocal immunofluorescence staining for SCF. *Scale bars: (A,B)* 50 μm; *(C–F)* 10 μm).

MCs have been implicated in the induction of angiogenesis (Meininger and Zetter, 1992), and increased MC numbers are often documented in conditions in which blood vessels proliferate (Kessler *et al*, 1976). Quantitation of vessel numbers associated with MC hyperplasia in human foreskin xenografts failed to reveal a significant angiogenic response in our model. Because normal MCs produce factors such as basic fibroblast growth factor and heparin known to provoke angiogenesis (Gruber *et al*, 1994), it is possible that the hyperplastic MCs in xenografts are deficient in these molecules. Alternatively, active and ongoing degranulation may be required for induction of a significant angiogenic response. Because we have shown the hyperplastic populations of MCs in xenografts to be secretagogue-responsive (Christofidou-Solomidou *et al*, 1996), a model now exists to more critically address this important biologic issue.

Recent data (Longley *et al*, 1996) indicate that MCs in some cases of human mastocytosis are clonal, with permanent phosphorylation and activation of the KIT receptor tyrosine kinase. Such cells are self-replicating and appear to escape normal regulatory mechanisms of MC homeostasis. Accordingly, it will be important to now determine whether clonal and autonomous MC proliferation exists or is inducible in human skin xenografts, as appears to develop in naturally occurring human mastocytosis.

In summary, we have developed a model for human cutaneous mast cell hyperplasia using neonatal foreskin xenografted to SCID mice. This system should provide useful information concerning mechanisms that govern MC kinetics and the chronic effects of increased MC numbers on the dermal and epidermal microenvironment. Therapeutic strategies such as topical corticosteroids (Lavker and Schechter, 1985) for the treatment of mast cell proliferative disorders may now also be tested and their mechanisms better understood.

This work was supported by Grants HL 49591 and CA 40358 from the National Institute of Health. We wish to thank Evgenia Argyris and Mildred Daise for their excellent technical assistance.

REFERENCES

Bosma GC, Custer RP, Bosma MJ: A severe combined immunodeficiency mutation in mouse. *Nature* 301:527–530, 1983

Caufield JP, El-Lati S, Thomas G, Church MK: Dissociated human foreskin mast cells degranulate in response to anti-IgE and substance P. *Lab Invest* 63:502–507, 1990

Chomszynski P, Sacchi N: Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chlorophorm extraction. *Anal Biochem* 162:156–9, 1987

Christofidou-Solomidou M, Albelda SM, Bennett FC, Murphy GF: Experimental production and modulation of human cytotoxic dermatitis in human-murine chimeras. *Am J Pathol* 150:631–639, 1997

Christofidou-Solomidou M, Murphy GF, Albelda SM: Induction of E-selectin-dependent leukocyte recruitment by mast cell degranulation in human skin grafts transplanted on SCID mice: *Am J Pathol* 148:177–188, 1996

Costa JJ, Demetri GD, Harrist TJ, Dvorak AM, Hayes DF, Merica EA, Menchaca DM, Gringeri AJ, Schwartz LB, Galli SJ: Recombinant human stem cell factor (Kit ligand) promotes human mast cell and melanocyte hyperplasia and functional activation *in vivo*. *J Exp Med* 183:2681–2686, 1996

Dvorak AM, Schleimer RP, Schulman ES, Lichtenstein LM: Human mast cells use conservation and condensation mechanisms during recovery from degranulation. *In vitro* studies with mast cells purified from human lungs. *Lab Invest* 54:663–678, 1986

Galli JG, Tsai M, Wershil BK: The c-kit receptor, stem cell factor, and mast cells. What each is teaching us about the others. *Am J Pathol* 142:965–974, 1993

Gilliam AC, Whitaker-Menezes D, Korngold R, Murphy GF: Apoptosis is the predominant form of epithelial target cell injury in acute experimental graft-versus-host disease. *J Invest Dermatol* 107:377–383, 1996

Grichnick JM, Crawford J, Jimenez F, Kurtzberg J, Buchanan M, Blackwell S, Clark RE, Hitchcock MG: Human recombinant stem-cell factor induces melanocytic hyperplasia in susceptible patients. *J Am Acad Dermatol* 33:577–583, 1995

Gruber, BL, Marchese, MJ, Kew, R: Transforming growth factor-β1 mediates chemotaxis of mast cells. *J Immunol* 152:5860–5865, 1994

Inemura A, Tsai M, Ando A, Wershil BK, Galli SJ: The c-kit ligand, stem cell factor, promotes mast cell survival by suppressing apoptosis. *Am J Pathol* 144:321–328-335, 1994

Juhasz I, Albelda SM, Elder DE, Murphy GF, Adachi K, Herlyn D, Valyi-Nagy I, Herlyn M: Growth and invasion of human melanomas in human skin grafted to immunodeficient mice. *Am J Pathol* 143:525–537, 1993c

Juhasz I, Lazarus GS, Murphy GF, Shi I-M, Herlyn M.: Development of pemphigus

vulgaris-like lesions in (SCID) mice reconstituted with lymphocytes from patients. *J Clin Invest* 92:2401–2407, 1993a

Juhasz I, Murphy GF, Herlyn M, Albelda SM: Regulation of extracellular matrix proteins and integrin cell substratum adhesion receptors on epithelium during cutaneous human wound healing in-vivo. *Am J Pathol* 143:1458–1469, 1993b

Kaminer MS, Lavker RM, Walsh LJ, Whitaker D, Zweiman B, Murphy GF: Extracellular localization of human connective tissue mast cell granule contents. *J Invest Dermatol* 96:1–8, 1991

Karnovsky MJ: A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. *J Cell Biol* 27:137–146, 1965

Kessler, DA, Langer, RS, Pless, NA, Folkman, J: Mast cells and tumor angiogenesis. *Int J Cancer* 18:703–708, 1976

Lavker RM, Schechter NM: Cutaneous mast cell depletion results from topical corticosteroid usage. *J Immunol* 135:2368–2373, 1985

Longley J: Is mastocytosis a mast cell neoplasia or a reactive hyperplasia? Clues from the study of mast cell growth factor. *Ann Med* 26:115–116, 1994

Longley J, Ding TG, Cuono C, Durden F, Crooks C, Hufeisen S, Eckert R, Wood GS: Isolation, detection, and amplification of intact mRNA from dermatome strips, epidermal sheets, and sorted epidermal cells. *J Invest Dermatol* 97:974–9, 1991

Longley J, Morganroth G, Turrell L, Ding TG, Anderson D, Williams D and Halaban R: Altered metabolism of mast cell growth factor (c-kit ligand) in cutaneous mastocytosis. *N Engl J Med* 328:1302–1307, 1993

Longley J, Tyrrell L, Lu S-Z, Ma Y-S, Langley K, Ding T-G, Duffy T, Jacobs P, Tang LH, Modlin I: Somatic c-kit activating mutation in urticaria pigmentosa and aggressive mastocytosis: establishment of clonality in a human mast cell neoplasm. *Nat Genet* 12:312–314, 1996

Matsumura T, Yamanaka T, Hashizume S, Irie Y, Nitta K: Tissue dispersion, cell harvest and fluid suspension culture by the use of bacterial neutral protease. *Jpn J Exp Med* 45:377–382, 1975

Meininger, CJ, Zetter, BR: Mast cells and angiogenesis. *Cancer Biol* 3:73, 1992

Mekori YA, Oh CK, Metcalfe DD: The role of c-kit and its ligand, stem cell factor, in mast cell apoptosis. *Int Arch Allergy Immunol* 107:136–138, 1995

Mirowski G, Austen KF, Horan RF, Chiang L, Sheffer AL, Murphy GF: Characterization of cellular dermal infiltrates in human cutaneous mastocytosis. *Lab Invest* 63:52–62, 1990

Murphy GF: Lymphoproliferative tumors. In: *Dermatopathology*. W.B. Saunders, Philadelphia, 1995, pp 270–273

Petzelbauer P, Groger M, Kunstfeld R, petzelbauer E, Wolff K: Human delayed type hypersensitivity reaction in a SCID mouse engrafted with with human T cells and autologous skin. *J Invest Dermatol* 107:576–581, 1996

Schechter NM, Choi JK, Slavin DA, Deresienski DT, Shigetoshi S, Dong G, Lavker RM, Proud D, Lazarus GS: Identification of a chymotrypsin-like proteinase in human mast cells. *J Immunol* 137:962–970, 1986

Tsai M, Takeishi T, Thompson H, Langley KE, Zsebo KS, Metcalfe DD, Geissler EN, Galli SJ: Induction of mast cell proliferation, maturation, and heparin synthesis by the rat c-kit ligand, stem cell factor. *Proc Natl Acad Sci USA* 88:6382–6386, 1991

Valent P, Ashman LK, Hinterberger W, Eckersberger F, Majdic O, Lechner K, Bettelheim P: Mast cell typing: demonstration of a distinct hematopoietic cell type and evidence for immunophenotypic relationship to mononuclear phagocytes. *Blood* 73:1778–1785, 1989

Walsh LJ, Trinchieri G, Waldorf HA, Whitaker D, Murphy GF: Human dermal mast cells contain and release tumor necrosis factor-a which induces endothelial leukocyte adhesion molecule-1. *Proc Natl Acad Sci USA* 88:4220–4224, 1991

Weidner N, Austen KF: Evidence for morphologic diversity of human mast cells. An ultrastructural study of mast cells from multiple body sites. *Lab Invest* 63:63–72, 1990

Weiss RR, Whitaker-Menezes D, Longley J, Bender J, Murphy GFD: Human dermal endothelial cells express membrane-associated mast cell growth factor. *J Invest Dermatol* 104:101–106, 1995

Whitaker-Menezes D, Schechter NM, Murphy GF: Serine proteinases are regionally segregated within mast cell granules. *Lab Invest* 72:34–41, 1995

Yan H-C, DeLisser HM, Pilewski JM, Barone KM, Szklut PJ, Chang X-J, Ahern TJ, Langer-Safer P, Albelda SM: *J Immunol* 152:3052–3063, 1994

Yan H-C, Juhasz I, Pilewski J, Murphy GF, Herlyn M, Albelda SM.: Human/severe combined immunodeficient mouse chimeras. An experimental in-vivo model system to study the regulation of human endothelial cells — leukocyte adhesion molecules. *J Clin Invest* 91:986–996, 1993