

A Human-SCID Mouse Model for Allergic Immune Responses: Bacterial Superantigen Enhances Skin Inflammation and Suppresses IgE Production

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Chronic skin colonization with *Staphylococcus aureus* is a well-known feature in atopic dermatitis. The aim of this study was to develop a human-SCID mouse model to analyze the possible role of bacterial superantigens in human allergic immune responses under *in vivo* conditions. SCID mice were reconstituted with peripheral blood mononuclear cells (between 2 and 9×10^7 cells per mouse) from atopic dermatitis patients sensitized to house dust mite allergen (Der p). Total and Der p specific antibody production required the following conditions: (i) injection of Der p; (ii) presence of CD14⁺ antigen-presenting cells; and (iii) IL-4 as shown by the inhibitory effect of human soluble IL-4 receptor on immunoglobulin E production. This model was used to study the immunomodulatory effects of the superantigen staphylococcal enterotoxin B in comparison with Der p. In intraperi-

toneally reconstituted human-SCID mice, topical treatment was ineffective in inducing skin inflammation. Therefore, additionally to intraperitoneal transfer, peripheral blood mononuclear cells from atopic donors were also injected intradermally. Such reconstituted SCID mice were then exposed via the skin to either Der p, staphylococcal enterotoxin B, or a combination of both. Maximal effects on epidermal inflammation and dermal T cell infiltration were obtained with staphylococcal enterotoxin B and Der p. Staphylococcal enterotoxin B alone was less effective and Der p only stimulated dermal T cell infiltration. These findings support the hypothesis that bacterial superantigens can act as trigger factors in allergic skin inflammation. **Key words:** atopic dermatitis/house dust mite allergen/SEB. *J Invest Dermatol* 110:224–231, 1998

Staphylococcus aureus and streptococcus produce a large family of proteins known as superantigens, which include staphylococcal enterotoxins, e.g., staphylococcal enterotoxin B (SEB), staphylococcal toxic shock syndrome toxin-1, and streptococcal pyrogenic exotoxins (Marrack and Kappler, 1990; Mollick *et al*, 1993). Unlike conventional antigens, superantigens do not require processing by antigen-presenting cells, because they are stimulatory in their native state and engage a sizeable fraction of the T cell compartment via direct binding and stimulation of T cell receptor V β elements (Dellabona *et al*, 1990; Herman *et al*, 1991).

Atopic dermatitis (AD) represents a main manifestation of the atopic syndrome and is characterized as a chronic inflammatory skin disease associated with pruritus and elevated serum immunoglobulin E (IgE) levels. Local infiltration of activated CD4⁺ T cells is a hallmark in the immunopathogenesis of the disease (Zachary *et al*, 1985; Lever *et al*, 1987; Van Joost *et al*, 1992). Acute AD lesions are characterized by the presence of T cells producing classical type-2 cytokines including

interleukin (IL)-4 and IL-5 (Sager *et al*, 1992; van Reijssen *et al*, 1992). In chronic skin lesions, a shift from elevated IL-4/IL-5 production to decreased levels of IL-4 and increased production of interferon (IFN)- γ was observed (Grewe *et al*, 1994, 1995; Werfel *et al*, 1996). T cell activation is associated with an imbalance in cytokine production resulting in increased production of IL-4 and reduced production of IFN- γ (Reinhold *et al*, 1990; Renz *et al*, 1992b; Jujo *et al*, 1992). T cell immunity to aeroallergens such as house dust mite allergen (Der p) is thought to play an important role in the pathogenesis of AD: allergen-specific T cell lines and clones can be generated from dermal and epidermal skin lesions. Less than 3% of the infiltrating T cells are allergen-specific, however (Sager *et al*, 1992; Werfel *et al*, 1996). Superantigens may act as an additional trigger factor in the development of skin inflammation. *Staphylococcus aureus* can be isolated from skin lesions in about 95% of all AD patients as compared with about 5% of nonatopic individuals (Leyden *et al*, 1974); *S. aureus* on inflamed AD lesions without clinical superinfection can reach 10^7 colony forming units per square centimeter on lesional skin (Hauser *et al*, 1985); *S. aureus* colonization can cause severe exacerbation of the disease in many patients and anti-staphylococcal therapy is beneficial in severe cases (Leyden *et al*, 1974). Despite this circumstantial evidence, a clear causal relationship has not yet been established. In order to define the role of superantigens in allergy, an experimental *in vivo* model of a humanized SCID (hu-SCID) mouse was developed.

Mice homozygous for the *scid* mutation (SCID mice) are severely deficient in functional B and T lymphocytes. Thus, SCID mice are unable to reject allogeneic and xenogeneic tissue transplants. Several

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Abbreviations: AD, atopic dermatitis; Der p, house dust mite allergen; FEIA, Fluorescence-Enzyme-Immuno-Assay; hu-SCID, human-SCID mouse; IgE, immunoglobulin E; PBMC, peripheral blood mononuclear cells; SEB, staphylococcal enterotoxin B; sIL-4R, soluble interleukin 4 receptor.

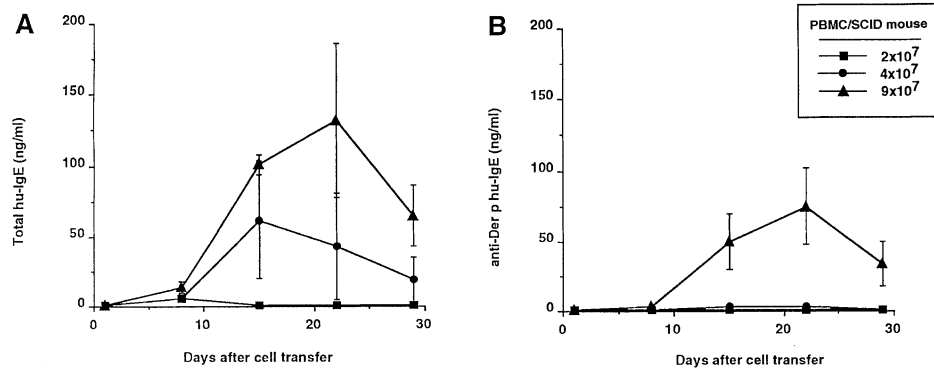


Figure 1. Dose and time dependent stimulation of hu-IgE antibody responses in hu-SCID mice. PBMC were prepared from one atopic donor (total IgE = 21.4 μ g per ml) who was sensitized to Der p as indicated by an anti-Der p IgE antibody concentration of 220 ng per ml and positive skin prick test reactions. Indicated cell numbers were i.p. injected into nine SCID mice together with Der p. On day 8, mice received an additional boost with Der p. Blood was drawn at indicated time points and total (A) and allergen-specific (B) hu-IgE antibody titers were determined by FEIA. The mean $\pm$ SD is expressed from three independently performed experiments.

studies report that SCID mice reconstituted with peripheral blood mononuclear cells (PBMC) from atopic patients sensitized to Der p, produced human-IgE when stimulated in the presence of adjuvant (Ito *et al.*, 1992; Pestel *et al.*, 1994; Spiegelberg *et al.*, 1994; Gagnon *et al.*, 1995). In models of immuno-competent mice we showed that allergen sensitization stimulated an allergic immune response in the absence of adjuvant with increased production of IgE (Renz *et al.*, 1992a). In this study we analyzed the conditions for human allergen-specific IgE production in reconstituted human SCID mice. We then examined the effect of topical aeroallergen and superantigen treatment on development of skin inflammation in this model.

MATERIALS AND METHODS

Study groups AD patients ($n = 13$) sensitized to Der p were identified by positive skin prick test reactions and elevated serum IgE antibody titers. Skin prick tests were performed with standard solutions obtained from ALK-Scherax (Hamburg, Germany). Human serum IgE antibody titers were measured by Fluorescence-Enzyme-Immuno-Assay (FEIA) (Pharmacia CAP System, Pharmacia, Uppsala, Sweden). Atopic patients responded to Der p with a wheal ≥ 10 mm in diameter and antibody titers ≥ 410 ng per ml for total IgE and ≥ 20 ng per ml for anti-Der p IgE. All patients suffered from severe AD based on the criteria by Hanifin and Rajka (1980). Non-atopic volunteers ($n = 4$) showed total serum IgE concentrations of ≤ 17 ng per ml and anti-Der p IgE of ≤ 0.8 ng per ml, and they had negative skin test reactions.

Animals SCID mice (C.B-17 *scid/scid*) were obtained from Bomholtgard (Ry, Denmark) and were maintained under pathogen-free conditions. Animals between 8 and 12 wk of age were screened for leakage by measurement of mouse IgG antibodies in the serum before the onset of experiments.

Preparation of PBMC Heparinized blood (200–250 ml) was collected from donors sensitized or nonsensitized to Der p and mononuclear cells were purified from heparinized blood by Lymphoprep (Nycomed, Oslo, Norway) density gradient centrifugation as previously described (Nasert *et al.*, 1996).

Reconstitution of SCID mice with human PBMC SCID mice were injected intraperitoneally (i.p.) with $2-9 \times 10^7$ PBMC together with 100 μ g purified house dust mite extract referred to as Der p (ALK-Scherax, Hamburg, Germany). The extract was calibrated based on the amount of Der p 1 and diluted in phosphate-buffered saline (PBS). One hundred micrograms of the extract contained 5 μ g Der p 1. Eight days later, SCID mice received an additional i.p. allergen boost (100 μ g Der p per injection). Between day 22 and day 28, total and allergen-specific human-IgE antibody titers in hu-SCID mice were measured by FEIA (Pharmacia CAP System).

Assessment of cytokine production after *in vivo* treatment with SEB SCID mice were injected i.p. with 9×10^7 PBMC together with Der p (100 μ g per injection), SEB (Serva-Toxin Technology, Heidelberg, Germany) (50 ng per injection), or a combination of both. Eight days later, SCID mice received an additional allergen or SEB boost or a combination of both, as described above. To assess cytokine production, human PBMC were recovered from reconstituted SCID mice by day 22. Human lymphocytes were collected

from the peritoneal cavity by washing with sterile, ice-cold PBS and resuspended after centrifugation (3000 rpm, 10 min) in PBS. Four $\times 10^6$ PBMC per well were incubated in the presence of concanavalin A (2.5 μ g per ml) for 48 h. Cell-free culture supernatants were harvested and production of IL-4, IL-5, and IFN- γ was analyzed by an enzyme-linked immunosorbent assay technique as described below.

Treatment of human-SCID mice with soluble IL-4 receptor (sIL-4R) Human PBMC reconstituted SCID mice were treated with human recombinant sIL-4R (150 μ g per mouse per application) or with murine sIL-4R (150 μ g per mouse per application). Human recombinant sIL-4R and murine sIL-4R were kindly provided by Dr. K. Enssle (Behringwerke, Marburg, Germany).

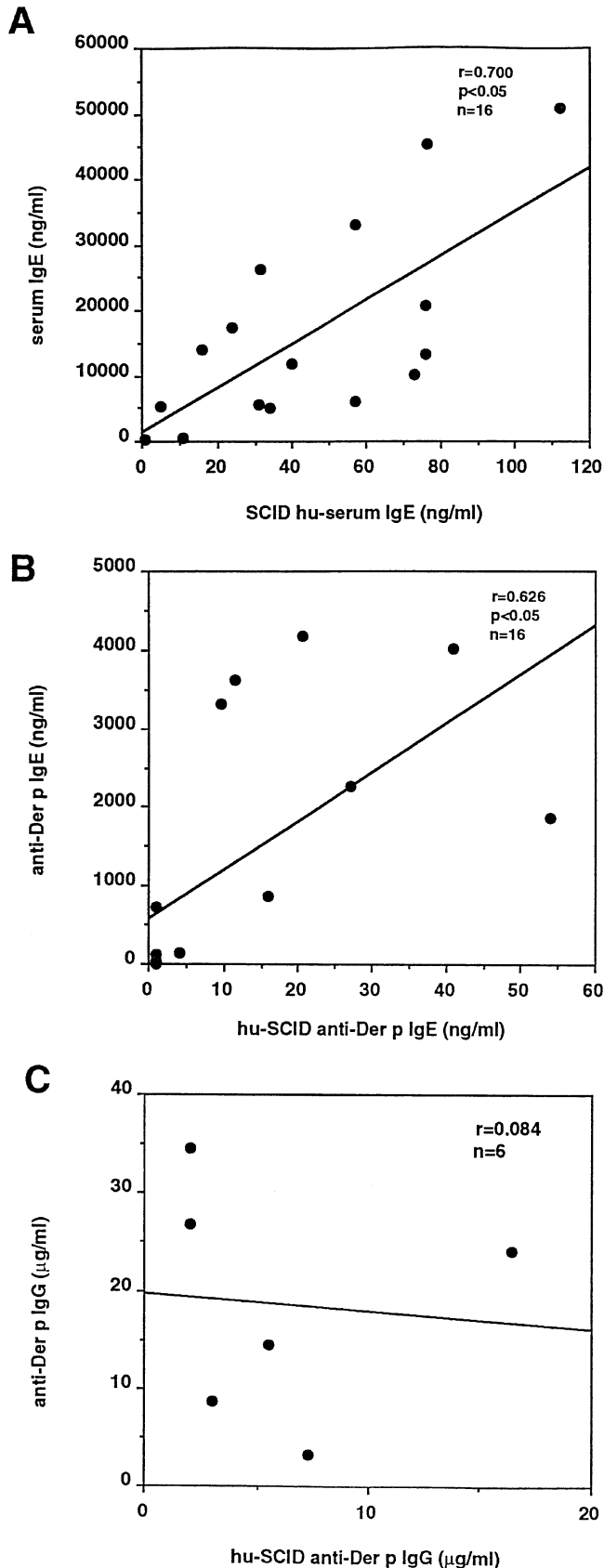
Topical treatment of SCID mice PBMC from AD patients or nonatopic individuals were injected i.p. (9×10^7 PBMC per mouse) together with Der p as described above. On day 14, 3×10^7 PBMC derived from the same donor were injected intradermally (i.d.). Every day between days 15 and 19 the epidermal lipid barrier was removed by scratching ($\times 10$) with 70% ethanol followed by topical application of 200 μ l solution containing either 100 μ g Der p per application or 50 ng SEB per application, or a mixture of both. As previously shown (Saloga *et al.*, 1994), and as also reproduced in this study, scratching alone did not cause a mechanic trauma. On day 20, histologic analysis of skin sections was performed.

Isolation and removal of CD14⁺ cell subset CD14⁺ antigen-presenting cells were removed from PBMC by multi-step cell purification protocol using immunomagnetic beads, and the purity of cells was assessed by flow cytometry using monoclonal antibodies against CD3, CD4, CD8, and IgE as previously described (Nasert *et al.*, 1996).

Culture assay for *in vitro* Ig production and cytokine production To assess immunoglobulin production, 4×10^6 PBMC per well were incubated in 24 well flat bottom tissue culture plates (Greiner, Solingen, Germany) diluted in tissue culture medium. Culture medium consisted of RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum (Behringwerke), 2 mM glutamine, 100 IU penicillin per ml, 100 μ g streptomycin (Biochrom, Berlin, Germany) per ml, and 0.2 μ g amphotericin B (Gibco BRL, Eggenstein, Germany) per ml. Cells were incubated with 700 ng Der p per ml, 50 ng SEB (Sigma, Deisenhofen, Germany) per ml, or a combination of both. After 10 d, cell free culture supernatants were harvested and IgE production was measured by FEIA (Pharmacia CAP System). To assess cytokine production, cell free culture supernatants were harvested after 48 h and analyzed for IL-4, IL-5, and IFN- γ production by enzyme-linked immunosorbent assay as previously described (Nasert *et al.*, 1996).

Histologic and ultrastructural evaluation Skin biopsies were prepared and fixed in 4% formaldehyde. Three micrometer paraffin-embedded sections were stained with hematoxylin and eosin or incubated with normal mouse serum followed by incubation with monoclonal antibodies directed either against human T lymphocytes (M-742, 1:100, Dako, Hamburg, Germany) or against human B lymphocytes (M-754, 1:20, Dako) for 30 min at 37°C. Immunostaining was performed by alkaline phosphatase:anti-alkaline phosphatase technique.

Negative controls using Tris-buffer instead of antibodies were free from immunolabeling. The dermal infiltrate was evaluated in a blinded fashion by an independent investigator. Three skin areas were randomly selected in each section and numbers of infiltrating human T cells were scored as <20 cells per mm^2 dermis, 20–100 cells per mm^2 dermis, or >100 cells per mm^2 dermis.



Statistical analysis Results are presented as mean values $\pm$ SD, unless otherwise stated. Mann-Whitney U test (exact test, single sided) was used to determine the level of difference between animal groups.

RESULTS

Production of human IgE in SCID mice reconstituted with PBMC from atopic patients SCID mice were reconstituted with PBMC from atopic and nonatopic individuals. **Figure 1** illustrates a representative dose response experiment performed with cells from one of three AD patients (total IgE 21,400 ng per ml; anti-Der p IgE 220 ng per ml). Transfer of at least 4×10^7 PMBC per mouse resulted in the production of total human-IgE antibody titers reaching maximal levels between day 14 and day 22. Induction of allergen-specific human-IgE required transfer of more than 4×10^7 PMBC per mouse. The kinetic of anti-Der p human-IgE antibody titers paralleled total human-IgE antibody titers peaking also around day 22.

Figure 2 compares total and allergen-specific IgE/IgG antibody titers from several sets of independently performed experiments. There was a positive correlation between total ($r = 0.700$) (**Fig 2A**) and allergen-specific ($r = 0.626$) (**Fig 2B**) human-IgE concentrations in the serum of the human donors and in reconstituted SCID mice. In contrast, no correlation was found between human allergen-specific IgG antibody titers in the serum of atopic cell donors and reconstituted hu-SCID mice.

To assess whether an allergen-specific IgE response could also be induced in mice reconstituted with nonatopic PBMC, cells were prepared from healthy nonatopic individuals (total IgE ≤ 17 ng per ml, anti-Der p IgE ≥ 0.8 ng per ml) and transferred into SCID mice as described earlier. After transfer of a comparable number of mononuclear cells, total IgE production never exceeded 4 ng per ml and allergen-specific IgE could not be detected. These results indicate that nonatopic PBMC could not be converted into IgE producing cells even when mice received an allergen challenge. Irrespective of whether SCID mice were reconstituted with PBMC from atopic or nonatopic cell donors, human-IgG antibodies were detectable on day 28 (1.9 ± 1.3 mg per ml), indicating that human T and B cells remained functional in SCID mice.

It was then determined whether the allergen challenge was required in order to mount an allergen-specific immune response in hu-SCID mice reconstituted with atopic PBMC. Although patients had high titers of circulating allergen-specific IgE antibodies, no anti-Der p specific antibodies could be detected in animals without allergen administration, indicating that production of specific IgE required continuous exposure to the allergen even in atopic patients (data not shown). Although most cell-donors were sensitized to a wide range of inhalant allergens as indicated by the presence of specific IgE antibodies to birch pollen allergen (**Fig 3**) and other allergens (data not shown), only anti-Der p IgE and not anti-birch pollen allergen IgE or IgG directed against other inhalant allergens was measurable in animals boosted with Der p (**Fig 3**).

It is possible that human-IgE production in reconstituted atopic SCID mice was caused by a recall of transferred human-IgE positive and/or memory B cells. In this case, removal of $\text{CD}14^+$ antigen-presenting cells should not disturb the capacity to mount an IgE antibody response. $\text{CD}14^+$ cells were removed from atopic PBMC by immunomagnetic beads technique and transferred into SCID mice. In parallel, SCID mice were treated with unfractionated PBMC derived

Figure 2. IgE titers in hu-SCID mice correlate with serum IgE of atopic patients. Human PMBC, prepared from atopic donors ($n = 12$) sensitized against house dust mite (Der p) (anti-Der p IgE ≥ 20 ng per ml), were transferred together with Der p (100 μg Der p per mouse) into the peritoneal cavity of SCID mice ($n = 16$, $30\text{--}90 \times 10^6$ PMBC per mouse). On day 8, mice received an additional injection of Der p. Blood was drawn between days 22 and 28 and total (A), anti-Der p IgE (B), and anti-Der p IgG (C) antibody titers were measured by FEIA (Pharmacia CAP System) in the serum and compared with the IgE/IgG antibody titers in the serum of the atopic donor. Each point represents serum IgE values of a patient and the corresponding serum IgE levels from SCID mice reconstituted with cells from the same donor.

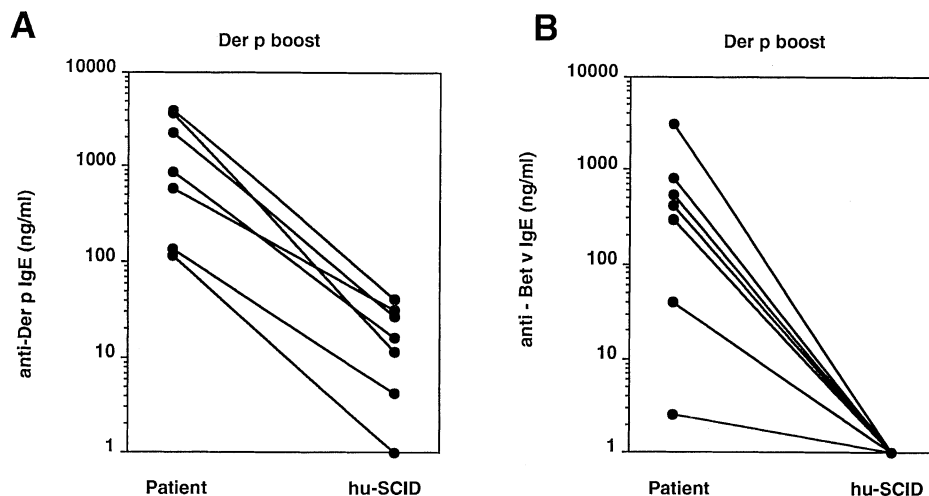


Figure 3. Induction of allergen-specific IgE depends on the allergen boost. SCID mice were reconstituted with 9×10^7 human PBMC per mouse as described in Fig 1. Anti-Der p IgE (A) and anti-birch pollen allergen IgE (B) antibody titers were measured by FEIA (Pharmacia CAP System). The allergen-specific IgE antibody titers in the serum of reconstituted SCID mice in comparison to the antibody titers of the human donors are indicated.

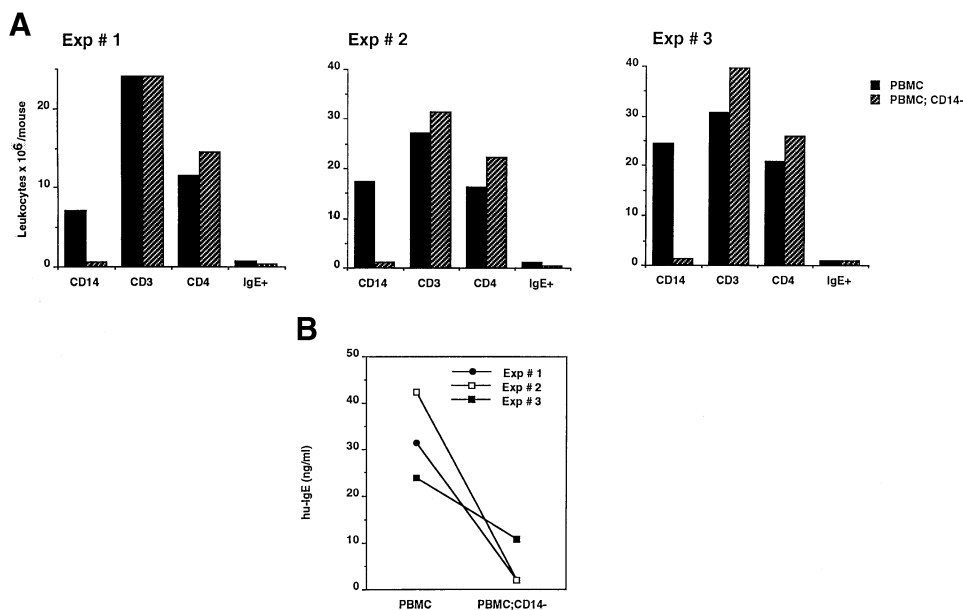


Figure 4. Induction of hu-IgE in SCID mice is dependent on CD14⁺ antigen-presenting cells. CD14⁺ antigen-presenting cells were separated from atopic PBMC by immuno-magnetic beads technique. Expression of CD14, CD3, CD4, and IgE on B cells was analyzed by fluorescence activating cell sorting analysis. SCID mice were reconstituted with human PBMC as described in Fig 1. Total human-IgE antibody titers were measured by FEIA (Pharmacia CAP System). Part (A) shows the distribution of cell subsets before and after CD14 depletion from three individual atopic patients that were transferred into SCID mice, and (B) represents total IgE antibody titers examined in reconstituted SCID mice on day 22.

from the same donor. Figure 4(A) illustrates the distribution of transferred cell populations indicating a similar distribution of CD3, CD4, and IgE positive cell subsets except for the presence of CD14⁺ cells. Figure 4(B) shows the corresponding total human-IgE antibody titers of these animals 22 d after transfer. The results indicate that in the absence of CD14⁺ antigen-presenting cells, human-IgE antibody synthesis was markedly reduced, suggesting an important role of CD14⁺ cell subsets in IgE production even from cell donors that were sensitized to this allergen.

IL-4 induces human B cell maturation and IgE synthesis in hu-SCID mice Because IgE production is known to depend to a large extent on the production of IL-4, we examined whether treatment with an IL-4 binding protein (recombinant human sIL-4R) would inhibit allergen induced IgE production *in vivo*. In a "prophylactic" approach, treatment was started at the time of cell transfer and continued throughout the experimental protocol (on days 1, 2, and 3, and again on days 7, 8, and 9). In a second protocol, "therapeutic" approach hu-

SCID mice were treated with human sIL-4R between days 9 and 13 only. Control hu-SCID mice were treated with recombinant murine sIL-4R, a protein that does not bind to human IL-4 (Rudolphi *et al*, 1993). "Prophylactic," but not "therapeutic," treatment with human-sIL-4R resulted in reduced levels of human-IgE antibody titers (Table I). These findings suggest that in this system IgE could be inhibited only when sIL-4R treatment was started early; however, sIL-4R was not sufficient to completely prevent production of IgE in all recipient mice.

In vivo SEB treatment reduces allergen-induced IgE synthesis Because this animal system seems to reproduce events that contribute to increased IgE antibody production in humans, we used it to assess the immunomodulatory role of superantigen in triggering T cell functions under *in vivo* conditions. As shown above, transfer of PBMC from AD patients followed by i.p. challenge with Der p resulted in measurable total IgE levels (Table II). Significantly lower levels of human-IgE production were detected when mice were

Table I. Prophylactic but not therapeutic treatment with hu-sIL-4R inhibits hu-IgE production in hu-SCID mice^a

Treatment	Atopic donor	Treatment of hu-PBMC SCID mice with:	Total hu-IgE (ng per ml)
Prophylactic	01	mu-sIL-4R	147.6
		hu-sIL-4R	6.4 (4%) ^b
	02	mu-sIL-4R	76.3
		hu-sIL-4R	50.4 (66%)
Therapeutical	10	mu-sIL-4R	56.9
		hu-sIL-4R	52.9 (93%)
	11	mu-sIL-4R	49.6
		hu-sIL-4R	45.5 (92%)
	12	mu-sIL-4R	72.7
		hu-sIL-4R	80.4 (111%)

^aNine $\times 10^7$ human PBMC derived from five atopic donors (total IgE ≥ 5000 ng per ml) were injected in combination with Der p into the peritoneal cavity of SCID mice as described in *Materials and Methods*. Eight days later hu-SCID mice received an additional allergen boost with Der p. Two mice were reconstituted with cells from the same donor. One mouse was then treated with murine (mu) recombinant sIL-4R (150 μ g per application), the other received human (hu) recombinant sIL-4R (150 μ g per application) by intraperitoneal injection. Two strategies of sIL-4R treatment were performed. In a "prophylactic" approach, mice were treated on days 1, 2, and 3, and again on days 7, 8, and 9. In the "therapeutic" protocol, mice were treated on days 9, 10, 11, 12, and 13 only. On day 22 total human-IgE antibody titers in hu-PBMC SCID mice were determined by FEIA.

^bPercentage of hu-IgE production in hu-sIL-4R treated mice compared with treatment with control protein (mu-sIL-4R, calculated as 100%).

Table II. Immunoglobulin and cytokine production in hu-SCID mice and AD patients^a

		Immunoglobulin and cytokine production in response to:		
		Der p	SEB	Der p + SEB
<i>in vivo</i>	total IgE (ng per ml)	298 $\pm$ 203 ^b	49 $\pm$ 39	7 $\pm$ 6*
	anti-Der p IgG (μ g per ml)	4.5 $\pm$ 2.2	4.3 $\pm$ 4.0	$\leq 2^*$
<i>ex vivo</i> ^c	IL-4 (pg per ml)	≤ 10	≤ 10	18.6 $\pm$ 4.5*
	IL-5 (pg per ml)	≤ 30	≤ 30	44.5 $\pm$ 6.4*
	IFN- γ (pg per ml)	≤ 100	140 $\pm$ 5	240 $\pm$ 1*
<i>in vitro</i> ^d	IL-4 (pg per ml)	≤ 10	22 $\pm$ 9	13 $\pm$ 3*
	IL-5 (pg per ml)	≤ 40	778 $\pm$ 174	534 $\pm$ 82*
	IFN- γ (pg per ml)	≤ 30	4706 $\pm$ 2240	3410 $\pm$ 2292*

^aNine $\times 10^7$ PBMC derived from two AD patients were injected intraperitoneally in combination with Der p (n = 3), SEB (n = 3), or both (n = 3); 8 d later mice received an i.p. booster injection with Der p, SEB, or both as described in *Materials and Methods*. Blood was drawn on day 22 and total and allergen-specific IgE antibody titers were measured.

^bResults are shown as mean $\pm$ SD. Der p treated *versus* Der p + SEB treated group, *p ≤ 0.05 (Mann-Whitney U test).

^cAfter 22 d human PBMC were recovered from reconstituted SCID mice and cultured for 48 h in the presence of Concanavalin A (2.5 μ g per ml). IL-4, IL-5, and IFN- γ production was measured in cell-free culture supernatant.

^dBefore transfer into SCID mice 4×10^6 PBMC derived from six AD patients were cultured in the presence of Der p (5 μ g per ml), SEB (20 ng per ml), or both for the determination of *in vitro* cytokine production. Cell-free culture supernatants were collected after 48 h and cytokine production was measured.

Table III. Effect of intraperitoneal and intradermal cell transfer on development of Der p- and SEB-induced skin inflammation

Cell transfer ^a			Topical treatment ^b	Epidermal changes	Dermal T cell infiltrate (cells per mm ² dermis)
No. of exp.	i.p.	i.d.			
n = 4	—	—	Der p + SEB	—	<20
n = 4	+	—	Der p + SEB	—	<20
n = 4	—	+	Der p + SEB	acanthosis	20–100
n = 3	+	+	Der p + SEB	acanthosis, parakeratosis, spongiosis ^c	>100

^aSCID mice received either PBMC from the same AD patient or PBS i.p. and i.d. as described in *Materials and Methods*.

^bBetween days 15 and 19 all mice were topically treated with a solution of Der p plus SEB as described in *Materials and Methods*. On day 20, animals were sacrificed, skin sections were performed and epidermal changes and dermal T cell infiltrate were evaluated as described in *Materials and Methods*.

^cIn two experiments, acanthosis, spongiosis, and parakeratosis were observed, whereas acanthosis alone was found in one experiment.

Table IV. Effect of Der p and SEB on development of skin inflammation

Cell transfer ^a			Topical treatment ^b	Epidermal changes	Dermal T cell infiltrate (cells per mm ² dermis)
No. of exp.	i.p.	i.d.			
n = 3	+	+	PBS	—	<20
n = 4	+	+	Der p	—	20–100
n = 4	+	+	SEB	acanthosis, parakeratosis, spongiosis	20–100
n = 3	+	+	Der p + SEB	acanthosis, parakeratosis, spongiosis	>100

^aSCID mice received PBMC from the same AD patient i.p. (day 1) and i.d. (day 14). Between days 15 and 19 all mice were topically treated with PBS, Der p, SEB, or a combination of Der p plus SEB as described in *Materials and Methods*. On day 20, animals were sacrificed, skin sections were hematoxylin and eosin stained or immunolabeled with an antibody directed against human T cells. Epidermal changes and dermal T cell infiltrate were evaluated as described in *Materials and Methods*.

reconstituted with cells from the same donor and i.p. treated with SEB and Der p. In addition, there was a tendency for levels of IgE to be lower in mice treated with SEB than in those treated with Der p alone. Anti-Der p IgG synthesis was not affected in animals treated with SEB. In contrast, anti-Der p IgG synthesis was significantly reduced (p < 0.001) in animals treated with SEB and Der p. To determine underlying causes of this effect, cytokine production was

assessed *in vitro* with human cells recovered by peritoneal lavage. Pilot experiments indicated that the cells required additional *in vitro* stimulation with mitogen in order for any interleukin secretion in culture supernatant to be detectable (data not shown). As indicated in **Table II**, T cells from mice that received Der p did not produce IL-4 (≤ 10 ng per ml) when restimulated *ex vivo*. In contrast, SEB treatment of hu-SCID mice resulted in measurable IFN- γ production.

Furthermore, in Der p exposed mice treated with SEB we found a synergistic enhancing effect on IFN- γ synthesis and IL-5 production. A similar cytokine pattern was obtained when atopic PBMC were stimulated *in vitro* with Der p and/or SEB. SEB markedly enhanced IL-5 and IFN- γ production, with little effect on IL-4 production.

Topical SEB treatment induces cutaneous inflammation These data indicate that SEB treatment of hu-SCID mice with atopic lymphocytes caused a shift in immune responses with inhibition of IgE and, on the other hand, stimulation of IFN- γ and IL-5 production. Because these two cytokines were found upregulated in chronic inflammatory lesions (Hamid *et al*, 1994), we examined whether topical Der p and SEB treatment would trigger an allergic inflammatory response of the skin. Topical application was carried out according to a previously published protocol (Saloga *et al*, 1994). Epidermal and dermal skin inflammation were observed when i.p. reconstituted SCID mice received an additional intradermal injection of PBMC from the same AD donor 14 d before topical application of Der p plus SEB was started (Table III). In contrast, topical treatment of mice without cell injection or with either i.p. or i.d. cell injection did not produce an equivalent inflammatory response.

This model was then utilized to assess whether the superantigen and/or the allergen were responsible for these effects. For this purpose i.p. plus i.d. injected SCID mice from the same AD donor were topically treated with either PBS, Der p, SEB, or a combination of Der p and SEB. Application of PBS alone produced no pathologic changes in epidermis and only a few human T cells were observed in the dermis (<20 cells per mm² dermis) (Table IV). This result also confirms previous data (Saloga *et al*, 1994) that shows that shaving and scratching of the skin with 70% ethanol did not cause a mechanic trauma. Application of Der p to the skin triggered moderate dermal T cell infiltrate (20–100 cells per mm² dermis), without changes in the epidermis (Fig 5A, D). When SEB was applied to the skin a similar cellular infiltrate (20–100 cells per mm² dermis) was observed in the dermis. In addition the epidermis showed signs of acanthosis, parakeratosis, and spongiosis (Fig 5B, E). Following the simultaneous application of SEB and Der p, a marked human T cell infiltrate (>100 cells per mm² dermis) in the dermis was observed (Fig 5C, F). In addition to T cell infiltration, similar epidermal changes were observed as with SEB alone.

In order to examine whether this type of response depended on transfer of AD PBMC or could also be induced with nonatopic PBMC, nonatopic PBMC SCID mice were also treated topically with Der p plus SEB. The results from these experiments are summarized in Table V. In nonatopic PBMC SCID mice the epidermis remained normal and few human T cells (<20 cells per mm² dermis) were detected in the dermis. In contrast, AD PBMC SCID mice developed the type of skin inflammation described above (Fig 5C, F; Table IV).

DISCUSSION

In contrast to other models of hu-SCID mice of (allergen-specific) Ig antibody responses (Ito *et al*, 1992; Pestel *et al*, 1994; Spiegelberg *et al*, 1994; Gagnon *et al*, 1995), we set out to stimulate allergen-specific IgE antibody production in the absence of any adjuvant. Adjuvants such as aluminum hydroxide are known to provide nonspecific inflammatory signals (Holt *et al*, 1991) and it is difficult to distinguish between direct effects caused by the allergen itself and nonspecific potent immune-enhancing effects induced by adjuvants. In our *in vivo* model of human allergic immune responses, SEB was identified as a potent immune-modulator that promoted development of a cutaneous inflammatory response with simultaneous inhibition of systemic IgE production.

Several factors and components were found to be necessary for an allergen-specific antibody response in this hu-SCID model. First, allergen exposure was necessary to trigger IgE production, even when PBMC from patients with high IgE serum levels were transferred into SCID mice. Although IgE-positive B cells were present in the injected cell population, restimulation with allergen was necessary to trigger IgE production, because in the absence of allergen no IgE antibody titers were detected. In this hu-SCID mouse system, induction of

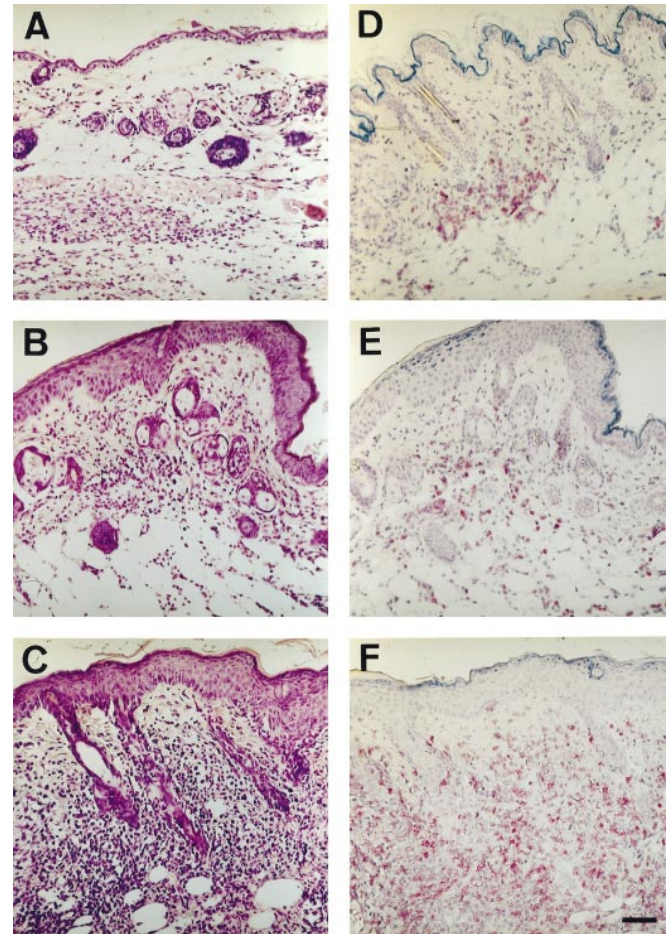


Figure 5. Topical superantigen triggers dermal T cell infiltration in hu-SCID mice. PBMC were prepared from an AD patient and injected i.p. (day 1) and i.d. (day 14) as described in *Materials and Methods*. Between days 15 and 19 a solution of Der p (A, D) (100 μ g Der p per application), SEB (B, E) (50 ng SEB per application), or a combination of both (C, F) was applied to the skin. One day after the last treatment, animals were sacrificed and skin histology was evaluated. (A, B, C) Hematoxylin and eosin staining; (D, E, F) immuno-histologic staining with anti-human T cell antibody as described in *Materials and Methods*. One of three similar experiments are shown. Scale bar, 100 μ m.

allergen-specific IgE was always paralleled by high titers of total IgE. In addition to allergen-specific IgE, enhancement of total IgE also required an allergen challenge and was not observed in the absence of allergen injections. The IgE antibody titers measured in SCID mice paralleled IgE titers of allergic cell donors. This suggests that the hu-SCID mouse model of human-IgE production induced by allergen challenges in the absence of adjuvant is suitable for the study of certain aspects of the allergic immune response.

The allergen-specific immune response involves CD14 positive antigen-presenting cells. It is assumed that antigen-presenting cells vary in their ability to induce type 1 *versus* type 2 responses: macrophages tend to favor the induction of type 1 cells (Gajewski *et al*, 1991). We initially hypothesized that B cells would be effective inducers of an allergen-specific (type 2) immune response, because it has been shown that B cells are much more effective at promoting IL-4 production than macrophages (Schmitz *et al*, 1993). Surprisingly, after removal of CD14 positive cells, IgE production was stimulated to a much lesser extent even when equal numbers of T and B cells were transferred from the same allergic cell donor into SCID mice. It is possible that *in vivo* allergen processing and presentation by macrophages and/or dendritic cells provide important costimulatory signals that could not be delivered by B cells. Although in two of the three donors the absolute numbers of IgE positive B cells were slightly reduced after

Table V. Effect of non-atopic PBMC on development of skin inflammation^a

Treatment			Topical treatment ^b	Epidermal changes	Dermal T cell infiltrate (cells per mm ² dermis)
No. of exp.	i.p.	i.d.			
n = 3	AD	AD	Der p + SEB	acanthosis, parakeratosis, spongiosis	>100
n = 4	Non-atopic	Non-atopic	Der p + SEB	—	<20

^aSCID mice received either PBMC from the same nonatopic or AD and were topically treated with Der p plus SEB as described in **Table IV**. On day 20, animals were sacrificed and epidermal changes and dermal T cell infiltration were evaluated as described in *Materials and Methods*.

CD14 depletion procedure, it is unlikely that this significantly affected *in vivo* IgE production, because it is known that a small number of IgE committed B cells is sufficient to mount IgE production in SCID mice (Pestel *et al*, 1994).

IL-4 represents an important cytokine that triggers the *in vivo* allergic immune response at least during the initial stages. In order to assess whether IL-4 was involved in the regulation of the allergic immune response, hu-SCID mice were treated with recombinant human soluble IL-4R, which binds specifically to human but not to murine IL-4 (Rudolph *et al*, 1993). It has recently been shown that sIL-4R inhibits several murine immune responses, including allergen-specific IgE/IgG1 production, development of immediate-type skin test responses, and prevention of increased airway responsiveness (Renz *et al*, 1995, 1996). Furthermore, the receptor downregulates human-IgE production *in vitro* (Nasert *et al*, 1995). The data presented in this study provide evidence that human sIL-4R also inhibits human-IgE production in hu-SCID mice; however, when the receptor was administered in a "therapeutic" treatment regimen hardly any reduction was observed compared with mice reconstituted with cells from the same patient and treated with murine sIL-4R as a control protein. Only when the receptor was administered at the time of cell transfer and continued throughout the experimental protocol was there an inhibitory effect; however, the suppression of IgE was never complete and residual IgE antibody titers remained. These findings support recent data suggesting that IL-4 is an early cytokine involved in IgE production, but other cytokines, including IL-13, also play an important role in IgE synthesis (Punnonen *et al*, 1993; McKenzie *et al*, 1993).

Because there is circumstantial clinical evidence that bacterial superantigens may trigger allergic inflammatory immune responses (reviewed in Hauser and Orbea, 1993; Leung, 1995), particularly in AD, this hu-SCID model was used to assess the effect of a prototypic superantigen (SEB) on IgE production, cytokine release, and cutaneous inflammation. If SEB contributes to allergic inflammation in AD patients, it should enhance IgE production by its capacity to stimulate "pro-allergic" T cells; however, experiments revealed an opposite effect, with low doses of SEB inhibiting IgE. To analyze the underlying mechanism, cytokine production was measured from AD lymphocytes prepared before and after transfer into hu-SCID mice. Freshly prepared lymphocytes from the same AD patients (**Table II**) responded with marked increases in IL-4, IL-5, and IFN- γ production when they were stimulated with SEB or a combination of SEB and Der p. Cells were then injected intraperitoneally and harvested after about 3 wk for analysis of their cytokine profile (**Table II**). *In vivo* treatment with SEB augmented Der p-induced IL-5 and IFN- γ production *in vitro*. These two cytokines play an important role in triggering allergic inflammatory immune responses (Hamid *et al*, 1994). IL-5 is required for eosinophil recruitment and survival, a cell population accumulating at the site of inflammation (Broide *et al*, 1992) and contributing to tissue damage associated with acute and chronic inflammation. On the other hand, IFN- γ not only counteracts many of the IL-4 mediated immune functions but also contributes to the development of inflammation by causing monocytes and macrophages to release several potent pro-inflammatory mediators.

In hu-SCID mice, recruitment of lymphocytes from the circulation to the skin and back again could be abnormal. SCID mice lack a lymphatic system, and it is still unclear how and to what extent human T cells interact with murine adhesion molecules on vascular endothelial cells. To bypass these limitations, Petzelbauer *et al* (1996) recently

reported on a hu-SCID model in which skin transplants provided an autologous environment for previously transplanted T cells. We used intradermal injection to bring human cells as close as possible to the site of reaction in sufficient numbers. The highest degree of skin inflammation was detected when a combination of both Der p and SEB was applied. Marked morphologic changes in epidermis (acanthosis, parakeratosis) were paralleled by T cell infiltration in the dermis. These morphologic changes reflect some of the histopathologic features in AD lesions (Murphy, 1995; Cohen *et al*, 1997). This implies a functional interaction between epidermally applied superantigens and allergens with the human T and B cell system in SCID mice. From our experiments, however, it is impossible to delineate the exact mechanism through which SEB activated the human T cells. It may bind to murine major histocompatibility complex class II molecules and allow bridging with human T cells, or, as human major histocompatibility complex class II expressing cells (activated T cells, monocytes, B cells) were simultaneously injected, autologous activation of human T cells would also be possible.

Taken together, these results demonstrate bi-directional *in vitro* effects of SEB treatment. SEB inhibited IgE production, but a marked pro-inflammatory effect was also observed following topical application on the skin. Thus, this model of hu-SCID mice allows the *in vivo* analysis of human allergic immune responses. Our results suggest that allergen exposure, presence of CD14⁺ antigen-presenting cells, and production of IL-4 contribute to human allergen-specific IgE production in the SCID model. This immune response can be altered by bacterial superantigen in a bi-directional fashion resulting in suppressed IgE production and enhanced local inflammatory immune reaction. Further studies using the hu-SCID model will examine strategies of specific immuno-intervention.

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REFERENCES

- Broide DH, Paine MM, Firestein GS: Eosinophils express interleukin 5 and granulocyte macrophage-colony-stimulating factor mRNA at sites of allergic inflammation in asthmatics. *J Clin Invest* 90:1414-1424, 1992
- Cohen LM, Kart Skopicki D, Harrist TG, Clark WH: In: Elder D (ed.). *Lever's Histopathology of the Skin*. Lippincott-Raven, New York, 1997, pp 209-252
- Dellabona P, Peccoud J, Kappler J, Marrack P, Benoist C, Mathis D: Superantigens interact with MHC class II molecules outside the antigen groove. *Cell* 62:1115-1121, 1990
- Gagnon R, Boutin Y, Hébert J: Lol p I-specific IgE and IgG synthesis by peripheral blood mononuclear cells from atopic subjects. *J Allergy Clin Immunol* 95:1268-1275, 1995
- Gajewski TF, Pinna M, Wong T, Fitch FW: Murine Th1 and Th2 clones proliferate optimally in response to distinct antigen-presenting cell populations. *J Immunol* 146:1750-1758, 1991
- Grewe M, Gyufko K, Schöpf E, Krutmann J: Lesional expression of interferon- γ in atopic eczema. *Lancet* 343:25-26, 1994
- Grewe M, Walter S, Gyufko K, Czech W, Schöpf E, Krutmann J: Analysis of the cytokine pattern expressed *in situ* in inhalant allergen patch test reactions of atopic dermatitis patients. *J Invest Dermatol* 105:407-410, 1995
- Hamid Q, Boguniewicz M, Leung DYM: Differential *in situ* cytokine gene expression in acute vs. chronic atopic dermatitis. *J Clin Invest* 94:870-876, 1994
- Hanifin JM, Rajka G: Diagnostic features of atopic dermatitis. *Acta Dermato Venereol* 60 (Suppl. 92):44-47, 1980
- Hauser C, Orbea A: Superantigens and their role in immune-mediated disease. *J Invest Dermatol* 101:503-505, 1993

- Hauser C, Wuehrich B, Matter L, Wilhelm JA, Sonnabend W, Schopfer K: Staphylococcus aureus skin colonization in atopic dermatitis patients. *Dermatologica* 170:35–39, 1985
- Herman A, Kappler JW, Marrack P, Pullen AM: Superantigens: mechanism of T cell stimulation and role in immune response. *Ann Rev Immunol* 9:745–772, 1991
- Holt PG, Rose AH, Batty JE, Turner KJ: Induction of adjuvant-independent IgE responses in inbred mice, primary, secondary and persistent IgE responses to ovalbumin and ovomucoid. *Int Arch Allergy Immunol* 65:42–50, 1991
- Ito M, Matsuzaki G, Uno S, Katakai Y, Suko M, Endo S, Okudaira H: Regulation of human IgE production by peripheral blood mononuclear cells from atopic patients in mice with severe combined immunodeficiency. *Int Arch Allergy Immunol* 99:373–376, 1992
- Jujo K, Renz H, Abe J, Gelfand EW, Leung DY: Decreased interferon gamma and increased interleukin-4 production in atopic dermatitis promotes IgE synthesis. *J Allergy Clin Immunol* 90:323–331, 1992
- Leung DY: Atopic dermatitis: The skin as a window into the pathogenesis of chronic allergic diseases. *J Allergy Clin Immunol* 96:302–319, 1995
- Lever R, Turbitt M, Sanderson A, MacKie R: Immunophenotyping of the cutaneous infiltrate and of the mononuclear cells in the peripheral blood in patients with atopic dermatitis. *J Invest Dermatol* 89:4–7, 1987
- Leyden JL, Marples RR, Kligman AM: Staphylococcus aureus in the lesions of atopic dermatitis. *Br J Dermatol* 90:525–530, 1974
- Marrack P, Kappler J: The staphylococcal enterotoxin and their relatives. *Sci* 248:705–711, 1990
- McKenzie ANJ, Culpepper JA, Waal Malefyt R, et al: Interleukin 13, a T cell-derived cytokine that regulates human monocyte and B cell function. *Proc Natl Acad Sci USA* 90:3735–3739, 1993
- Mollick JA, McMasters RL, Grossman D, Rich RR: Localization of a site on bacterial superantigens that determines T cell receptor beta chain specificity. *J Exp Med* 177:283–293, 1993
- Murphy G: In: *Dermatopathology*. WB Saunders, Philadelphia, 1995, pp 54–55
- Nasert S, Millner M, Herz U, Lack G, Wahn U, Gelfand EW, Renz H: Therapeutic interference with interferon- γ (IFN- γ) and soluble IL-4 receptor (sIL-4R) in allergic diseases. *Behring Inst Mitt* 96:118–130, 1995
- Nasert S, Burtchen N, Kussebi F, et al: Stimulation of IgE and IgA production by CD45RA T helper cells in atopic patients. *J Immunol* 157:441–448, 1996
- Pestel J, Jeannin P, Delneste Y, et al: Human IgE in SCID mice reconstituted with peripheral blood mononuclear cells from Dermatophagoides pteronyssinus-sensitive patients. *J Immunol* 153:3804–3810, 1994
- Petzeltbauer P, Gröger M, Kunstfeld R, Petzeltbauer E, Wolff K: Human delayed-type hypersensitivity reaction in a SCID mouse engrafted with human T cells and autologous skin. *J Invest Dermatol* 107:576–581, 1996
- Punnonen J, Aversa G, Cocks BG, et al: Interleukin 13 induces interleukin 4-independent IgG4 and IgE synthesis and CD23 expression by human B cells. *Proc Natl Acad Sci USA* 90:3730–3734, 1993
- Reinhold U, Wehrmann W, Kukul S, Kreysel HW: Evidence that defective interferon-gamma production in atopic dermatitis patients is due to intrinsic abnormalities. *Clin Exp Immunol* 79:374–379, 1990
- Renz H, Jujo K, Bradley KL, Domenico J, Gelfand EW, Leung DY: Enhanced IL-4 production and IL-4 receptor expression in atopic dermatitis and their modulation by interferon-gamma. *J Invest Dermatol* 99:403–408, 1992a
- Renz H, Smith HR, Henson JE, Ray BS, Irvin CG, Gelfand EW: Aerosolized antigen exposure without adjuvant causes increased IgE production and airway responsiveness in the mouse. *J Allergy Clin Immunol* 89:1127–1138, 1992b
- Renz H, Ennsle K, Lauffer L, Kurrle R, Gelfand EW: Inhibition of allergen-induced IgE and IgG1 production by soluble IL-4 receptor. *Int Arch Allergy Immunol* 106:46–54, 1995
- Renz H, Bradley K, Ennsle K, Loader JE, Larsen GL, Gelfand EW: Prevention of the development of immediate hypersensitivity and airway hyperresponsiveness following *in vivo* treatment with soluble IL-4 receptor. *Int Arch Allergy Immunol* 109:167–176, 1996
- Rudolph A, Ennsle KH, Claesson MH, Reimann J: Adoptive transfer of low numbers of CD4+ T cells into SCID mice chronically treated with soluble IL-4 receptor does not prevent engraftment of IL-4 producing T cells. *Scand J Immunol* 38:57–64, 1993
- Sager N, Feldmann A, Schilling G, Kreitsch P, Neumann C: House dust mite-specific T cells in the skin of subjects with atopic dermatitis: Frequency and lymphokine profile in the allergen patch test. *J Allergy Clin Immunol* 89:801–810, 1992
- Saloga J, Renz H, Larsen GL, Gelfand EW: Increased airways responsiveness in mice depends on local challenge with antigen. *Am J Respir Crit Care Med* 149:65–70, 1994
- Schmitz J, Assenmacher M, Radbruch A: Regulation of T helper cell cytokine expression: functional dichotomy of antigen-presenting cells. *Eur J Immunol* 23:191–199, 1993
- Spiegelberg HL, Beck L, Kocher HP, Fanslow WC, Lucas AH: Role of interleukin-4 in human immunoglobulin E formation in hu-PBL-SCID mice. *J Clin Invest* 93:711–717, 1994
- Van Joost T, Kozel MM, Tank B, Troost R, Prens EP: Cyclosporine in atopic dermatitis. Modulation in the expression of immunologic markers in lesional skin. *J Am Acad Dermatol* 27:922–928, 1992
- Van Reijssen FC, Bruijnzeel-Koomen CAFM, Kalthoff FS, Maggi E, Romagnani S, Westland JKT, Mudde GC: Skin-derived aeroallergen-specific T cell clones of Th2 phenotype in patients with atopic dermatitis. *J Allergy Clin Immunol* 90:184–192, 1992
- Werfel T, Morita A, Grewe M, Renz H, Wahn U, Krutmann J, Kapp A: Allergen specificity of skin-infiltrating T cells is not restricted to a type-2 cytokine pattern in chronic skin lesions of atopic dermatitis. *J Invest Dermatol* 107:871–876, 1996
- Zachary CB, Poulter LW, MacDonald DM: Cell-mediated immune response in atopic dermatitis: the relevance of antigen-presenting cells. *Br J Dermatol* 113 (Suppl. 28):10–16, 1985