

Keratinocyte-derived, CD80-mediated costimulation is associated with hapten-specific IgE production during contact hypersensitivity to T_H1 haptens

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Background: B7-1 transgenic mice exhibit exaggerated and persistent contact hypersensitivity responses compared with normal mice.

Objective: Because B7-1 and B7-2 deliver different costimulatory signals to T cells during antigen presentation, the purpose of this study was to compare B7-1 and B7-2 on keratinocytes and to compare their effects on contact hypersensitivity.

Methods: Contact hypersensitivity was studied in transgenic mice whose keratinocytes constitutively expressed B7-1, B7-2, or no costimulatory molecules (nontransgenic mice).

Results: B7-1 transgenic mice, and to a lesser extent B7-2 transgenic mice, developed exaggerated ear swelling responses after sensitization and challenge with haptens such as trinitrochlorobenzene or dinitrofluorobenzene. Ear swelling responses in B7-1 transgenic mice were characterized by the presence of markedly elevated inflammatory cytokine transcripts (IL-6, TNF- α , and lymphotoxin β) as well as IL-10 compared with either B7-2 or nontransgenic mice. Hapten-specific IgE was detected by ELISA in B7-1 transgenic mice but not B7-2 transgenic or nontransgenic mice. Only B7-1 transgenic mice exhibited significant immediate type ear swelling responses to the hapten trinitrochlorobenzene. In addition, their sera can passively transfer cutaneous anaphylaxis to naive C57BL/6 mice, indicating that the hapten-specific IgE was relevant to the immediate ear swelling responses.

Conclusion: These data suggest that keratinocyte-derived costimulation mediated by B7-1 but not B7-2 results in the emergence of T_H2-lymphocyte immune responses to T_H1 haptens. Because human keratinocytes have been noted to express B7-1–like molecules in certain inflammatory skin

diseases, this model may be important in understanding the pathophysiology of T_H2-lymphocyte-mediated skin diseases such as atopic dermatitis. (*J Allergy Clin Immunol* 2005;115:383-90.)

Key words: Contact dermatitis, keratinocytes, costimulation, IgE, transgenic mice

Epidermal keratinocytes play an important role in modulating cutaneous immune responses.¹⁻³ Antigen presentation by Ia⁺ keratinocytes to T-helper lymphocytes has been studied, and it has been demonstrated that for antigen and hapten-specific responses, keratinocytes do not activate T lymphocytes and are considered amateur antigen-presenting cells (APCs). Antigen presentation by keratinocytes to T-helper cells is not an immunologically null event, because keratinocytes induce clonal anergy in antigen-specific T_H1 lymphocytes.⁴⁻⁶ Thus, at least for some types of cutaneous immune responses, keratinocytes may play a role in maintaining peripheral tolerance by inducing clonal anergy in epidermotrophic T_H cells, counterbalancing the immunostimulatory effects of epidermal Langerhans cells.

Despite their tolerogenic APC properties, human keratinocytes have been demonstrated to express low levels of B7-1–like molecules as studied by immunocytochemistry, Northern blotting, and activation of gene reporter constructs.⁷⁻¹² As well, murine keratinocytes express low levels of B7-1 that can be detected by Western blotting in some strains of mice (A.A.G., unpublished data, 2003). In contrast, B7-2 has not been detected on human keratinocytes in either normal or inflamed skin.⁷⁻¹²

Providing cultured human keratinocytes with high levels of B7-1 (CD80; stable gene expression by transfection) can reverse this defective antigen presentation. Such B7-1–bearing Ia⁺ keratinocytes present alloantigens to resting T lymphocytes, resulting in proliferation and cytokine production.¹³ These data led to engineering of a keratin 14–recombinant DNA expression vector to target the expression of B7-1 in epidermal keratinocytes in transgenic mice.¹⁴⁻¹⁸ There is evidence that B7-1 and B7-2 derived from professional APCs do not deliver identical costimulatory signals to T cells during cell-cell interactions, with some literature suggesting a role for B7-2 in promoting T_H2-lymphocyte development.¹⁹⁻²⁴ Therefore,

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Abbreviations used

APC: Antigen-presenting cell
CHS: Contact hypersensitivity
RFU: Relative fluorescence unit

keratin 14–B7-2 transgenic mice were subsequently engineered to compare the effects of keratinocyte-derived B7-2 with those of B7-1 on the cutaneous immune response. B7-1 transgenic mice exhibit exaggerated and persistent ear swelling responses after hapten application,^{14,15} whereas there is no information on the effects of B7-2 expression by keratinocytes in transgenic mice. Herein we report the effects of B7-1 or B7-2 expression by keratinocytes in a CHS model.

METHODS**Mice**

Lines of keratin 14–B7-1 and keratin 14–B7-2 transgenic mice were generated as previously described.^{14,17} The keratin 14 promoter was used to drive expression of the B7-1 and B7-2 costimulatory molecules to basal keratinocytes.^{25,26} These transgenic mice were maintained in the C57BL/6 background. B7-1 and B7-2 transgenic mice were identified by immunohistochemistry of tail sections. The mice used in all experiments were 6 to 8 weeks old. All experimental procedures were reviewed and approved by the Committee on Animal Resources.

Confocal laser scanning microscopy to measure B7 expression in the epidermis of nontransgenic and transgenic mice

The relative levels of B7 expression were quantitated by using confocal laser scanning microscopy and computer-assisted data analysis of CTLA4/Ig-stained tail sections (Meridian Ultima Adherent Cell Analysis System and its accompanying Data Analysis System Master Program V3.32; Meridian Instruments, Okemos, Mich), as previously described.¹⁷

Contact hypersensitivity assay

Contact sensitivity responses to the haptens dinitrofluorobenzene and trinitrochlorobenzene were assayed. In all experiments, keratin 14–B7-1 transgenic, keratin 14–B7-2 transgenic, and nontransgenic mice that had been sensitized with the appropriate hapten were analyzed for contact hypersensitivity (CHS) as previously described by using dinitrofluorobenzene (Eastman Kodak, Rochester, NY) or trinitrochlorobenzene (ICN Pharmaceuticals, Plainville, NY).²⁷ Ear swelling was measured by using thickness gauge calipers (Swiss Precision Instruments, Carlstadt, NJ) at the time points indicated after challenge on the pinna of the ear. Data are reported as the mean change in ear thickness, ΔT = mean (thickness after challenge – baseline thickness), $\pm$ SD. All ear swelling assays were repeated on at least 3 separate occasions to assure reproducibility.

To amplify hapten-specific IgE responses in nontransgenic and transgenic mice, experimental mice were sensitized on the abdomen with dinitrofluorobenzene, followed by repeated elicitations as previously described.²⁸ One week after sensitization, allergic mice were challenged on the pinna of the ear 2 times per week (alternating the left and right ears) for a total of 3 weeks of repeated elicitations. One

week after the final elicitation, experimental mice were bled, and hapten-specific IgE was assayed.

RNase protection assay

Eight micrograms of total RNA was isolated from the ears by using standard methods²⁹ from sensitized mice before, 24 hours after, and 1 week after dinitrofluorobenzene challenge. The RiboQuant Multiprobe RNase Protection Assay System was used (mCK-3 Template Set; Pharmingen, San Diego, Calif).^{30,31} The protected RNA duplexes were resolved on a 40-cm, 6% 19:1 acrylamide sequencing gel. The dried gel was then used in several of rounds autoradiography such that exposure was within the linear recording range of the film (XOMAT; Eastman Kodak, Rochester, NY) for the cytokines of interest. Optical density readings of the resultant bands were obtained by using a Molecular Dynamics Personal Densitometer and Image-Quant v5.6 software (Molecular Dynamics, Sunnyvale, Calif). Specific cytokine transcripts were determined by comparing the relative migration of unprotected, undigested ³²P-labeled cytokine probes with that of RNase-digested, protected ³²P-labeled cytokine probes derived from a mouse mRNA standard set (EL-4 RNA). Hybridized, protected probes demonstrated specific, predictable alterations in their relative migration on the acrylamide gel.³⁰

RT-PCR

Total RNA isolated from the ears of sensitized mice before, 24 hours after, and 1 week after dinitrofluorobenzene challenge was used for RT-PCR with standard methods. The sequences for the primers were as follows: IL-10 upstream, 5'-AAG CTG AGA ACC AAG ACC CAG ACA TCA AGG CG-3'; downstream, 5'-AGC TAT CCC AGA GCC CCA GAT CCG ATT TTG G-3'; β -actin primers: upstream, 5'-GTG GGC CGC TCT AGG CAC CAA-3'; downstream, 5'-CTC TTT GAT GTC ACG CAC GAT TTC-3'. Specific cDNA obtained from RT was amplified by using previously described methods.¹⁸ An aliquot of 5 μ L of the PCR products was electrophoresed on a 1.5% agarose gel, then photographed.

Hapten-specific ELISA

To test sera from immunized mice for hapten-specific IgG, IgM, and IgE, a specific ELISA was developed by using standard techniques.³² ELISA plates (Corning Glass Works) were coated with 10 μ g trinitrophenyl-ovalbumin or dinitrophenyl-ovalbumin (Sigma Chemical, St Louis, Mo), and the plates were blocked with 0.25% BSA in Tween/PBS. Sera were then plated in triplicate to allow for antibody binding. Unbound antibody was washed off, and biotinylated antimouse IgM, IgG, and IgE (BD Pharmingen, San Diego, Calif) were added at 2 μ g/mL. The level of trinitrophenyl-specific or DNP-specific IgE was then analyzed by adding streptavidin-horseradish peroxidase at a dilution of 1:2000. *o*-Phenylenediamine dihydrochloride (Sigma Chemical Co) was added according to Sigma instructions for color development. Plates were read on a BioRad benchmark microplate reader (BioRad Laboratories, Hercules, Calif), and optical density was read at 570 nm.

Statistical analysis

Data were analyzed for statistical significance by using ANOVA (Statview Student Software Program; Abacus Concepts, Berkeley, Calif). Probability values of $<.05$ were considered statistically significant.

Passive transfer of cutaneous anaphylaxis and inhibition

Sera from naive or DNFB-sensitized mice were collected 3 weeks after sensitization from nontransgenic, B7-1 transgenic, and B7-2

transgenic mice. The sera were then administered via intradermal injection into the pinna of the ear (10 μ L for each side of the ear) of recipient naive, C57BL/6 mice (5 mice in each group). Twenty-four hours after the passive transfer of sera, baseline ear thickness was measured. Animals were then challenged on the pinna of the ear with 0.2% dinitrofluorobenzene. Ear swelling was measured at 20 minutes, and change in ear thickness was then calculated.

Mouse sera from B7-1 transgenic mice were preincubated with Dynalbeads M-280 Tosylactivated (Dynal Corp, Oslo, Norway) that had been coupled with monoclonal rat antimouse IgE (clone R35-72; BD Pharmingen) or monoclonal rat antimouse IgG1 (clone A85-3; BD Pharmingen) by using the manufacturer's suggested protocol. After a 24-hour incubation, the beads were removed from the sera by using magnetic separation. Untreated, anti-IgE-treated, or anti-IgG1-treated sera were then passively transferred to naive mice as described, followed by hapten challenge, and immediate ear swelling was then measured.

RESULTS

Analysis of total B7 expression levels on B7-1 transgenic and B7-2 transgenic mouse epidermal cells

To investigate the differences between B7-1-mediated and B7-2-mediated signaling during the course of cutaneous CHS reactions by using our transgenic mouse lines, it was necessary to confirm that the chosen B7-1 transgenic and B7-2 transgenic lines expressed equivalent levels of surface B7 molecules.

The fluorescence profiles obtained from the B7-1 transgenic and B7-2 transgenic samples show them to express similar amounts of CTLA4/Ig binding (B7-1 transgenic mean, 1200 relative fluorescence units [RFU]; B7-2 transgenic mean, 1000 RFU), which is significantly higher than the nontransgenic control sample (mean, 100 RFU). These data are representative of the results obtained from analysis of 3 independent members of each of the 3 groups.

Keratinocyte B7-1, but not B7-2, significantly enhances the amplitude and duration of CHS. We have previously shown that the presence of the B7-1 molecule on transgenic mouse keratinocytes leads to exaggerated and persistent CHS responses.^{14,15} These findings were independently confirmed.³⁶ To compare the abilities of B7-1 and B7-2 to enhance the cutaneous delayed type hypersensitivity response, groups of nontransgenic, B7-1-transgenic, and B7-2-transgenic mice ($N = 5$ /group) were sensitized with 0.5% dinitrofluorobenzene on days 0 and 1. Five days later, these animals were challenged with a single dose of 0.2% dinitrofluorobenzene on the pinna of the ear, and caliper measurements of the change in ear thickness from prechallenge baseline measurements were taken for each mouse at timed intervals. Analysis of ear swelling at 24-hour intervals showed that the ear swelling in the B7-1 transgenic mice was consistently exaggerated compared with that of the nontransgenic control mice (Fig 1, A). This was noted for the entire 2-week time course of the experiment and was statistically greater at the 3, 4, and 5 through 14-day time points ($P < .01$; Fig 1, A). In contrast with the data generated by

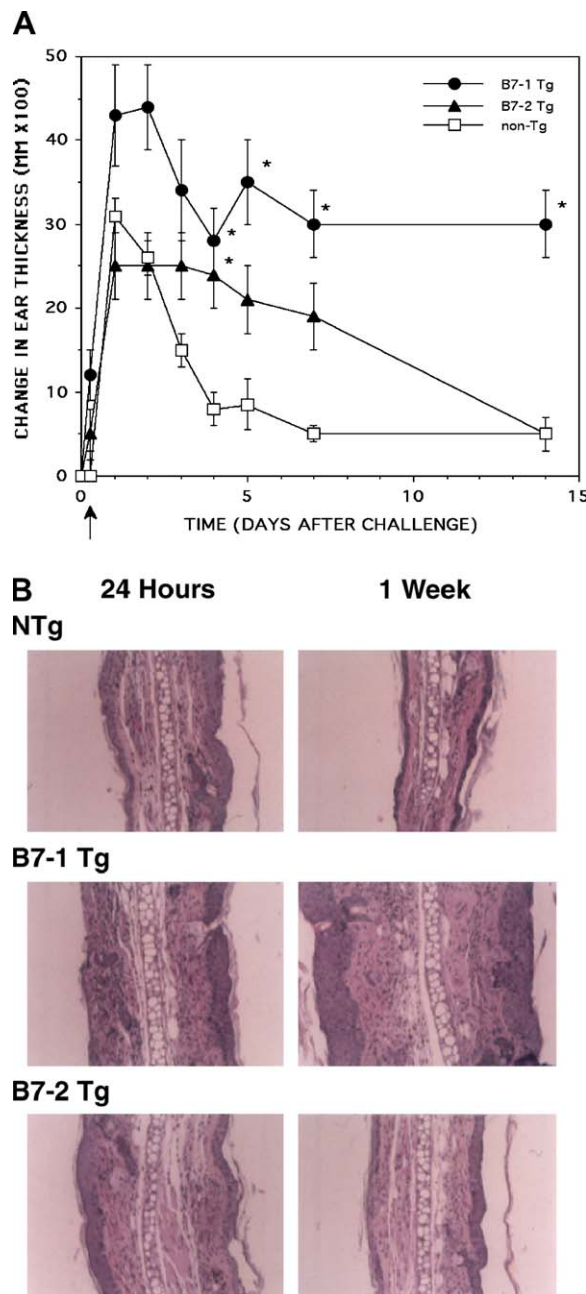


FIG 1. B7-1 transgenic (Tg) mice display exaggerated and persistent CHS response to the hapten dinitrofluorobenzene. **A**, Time course of ear swelling in hapten-sensitized mice. Arrow indicates first time point after challenge (6 hours; open squares, nontransgenic [NTg] mice; filled circles, B7-1 Tg mice; filled triangles, B7-2 Tg mice; mean $\pm$ SD; * $P < .05$ compared with either the nontransgenic or the B7-2 Tg mice). **B**, Skin biopsy specimens from CHS-involved ears were taken at 24 hours and 1 week after hapten challenge in the 3 different lines of mice.

the B7-1 transgenic mice, CHS in B7-2 transgenic mice was found to be moderately exaggerated, statistically greater ($P < .05$) than that of the control group at 4 days, but to display kinetics of resolution similar to those of the nontransgenic control group. When compared with the

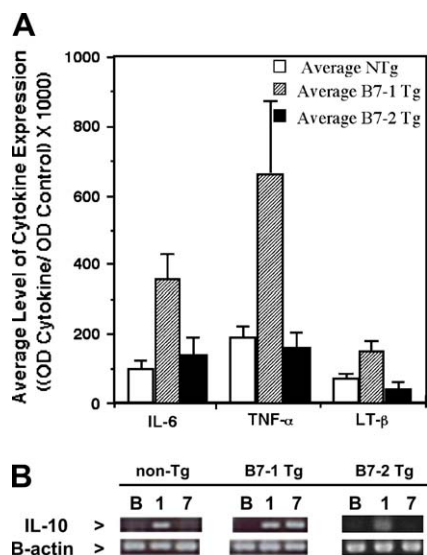


FIG 2. B7-1 transgenic (Tg) mice exhibit exaggerated production of proinflammatory cytokines TNF- α , IL-6, and lymphotoxin (LT) after hapten challenge. RNase protection assay to detect cytokine transcripts in mouse ear skin. **A**, Densitometry analysis (non-transgenic [NTg] mice, keratin 14-B7-1 Tg mice, or keratin 14-B7-2 Tg; mean $\pm$ SD of pooled optical density [OD] ratios of cytokine/control RNA [3/group]; * P < .05); **B**, RT-PCR analysis of IL-10 and β -actin transcripts in mouse skin (baseline [b], 1 day [1], and 7 days [7]) after hapten challenge in mice allergic to trinitrochlorobenzene.

B7-2 transgenic mice, the B7-1 transgenic mice exhibited statistically greater ear swelling responses at the 5 through 14-day time points (P < .05; significant). When the ear swelling responses to the hapten trinitrochlorobenzene were studied, similar patterns of kinetics and amplitude of the ear swelling responses were observed in these 3 groups of mice (data not shown). These data indicate that B7-1 and B7-2 differ in their abilities to elicit a chronic inflammatory reaction in the cutaneous environment.

Keratinocyte B7-1, not B7-2, induces histologic chronic inflammation. Histologic study of the pinna of the ear (CHS challenge site) at 24 hours and 1 week after hapten challenge in dinitrofluorobenzene-sensitized non-transgenic and B7-1 and B7-2 transgenic mice confirmed the ear swelling experiment. That is, skin biopsy specimens from B7-1 transgenic mice revealed enhanced inflammatory changes at 24 hours (denser inflammatory cell infiltrate of monocytes, lymphocytes, neutrophils, pronounced dermal edema, vasodilation, and epidermal hyperplasia) compared with B7-2 and nontransgenic mice (Fig 1, B). At 1 week, the histologic changes associated with CHS inflammation resolved in B7-2 and nontransgenic mice, but not in the B7-1 transgenic mice, in which there was persistent dermal inflammatory infiltrate, dermal edema, vasodilation, and epidermal hyperplasia.

Keratinocyte B7-1, but not B7-2, enhances the expression of inflammatory cytokine genes during CHS. After discovery that the B7-1 and B7-2 costimulatory molecules provide qualitatively different signals in the CHS response, gene expression associated with CHS was studied by using an RNase protection assay. At 24 hours after

hapten challenge in mice with allergy, there were significantly greater steady-state levels of 3 inflammatory cytokines—IL-6, TNF- α , and lymphotoxin β —in tissues from B7-1 transgenic mice compared with B7-2 or nontransgenic mice (Fig 2, A). The expression of these inflammatory cytokine genes persisted at 1 week in the B7-1 transgenic mice and became undetectable in the B7-2 and nontransgenic mice. Other cytokine genes were also examined in the RNase protection assay: there was no significant difference in IFN- γ gene expression among the 3 groups of experimental mice (data not shown). Other cytokine genes included in this RNase protection assay were IFN- β , TGF- β 1, and TGF- β 2, none of which were detected in the skin of any of the experimental mice (data not shown).

The immunomodulating cytokine IL-10 was persistently increased in mouse ear skin only in B7-1 transgenic mice (present at 1 day and 7 days after trinitrochlorobenzene challenge; Fig 2, B); in nontransgenic and B7-2 transgenic mice, IL-10 transcripts were transient (present at 1 day but not 7 days after trinitrochlorobenzene challenge).

Keratinocyte B7-1 results in hapten-specific IgE production. To determine whether keratinocyte-derived B7-1 or B7-2 modulated T-cell help for antihapten antibody production, sera from mice sensitized with either trinitrochlorobenzene or dinitrofluorobenzene were studied for the presence of hapten-specific IgE by using a specific ELISA (Fig 3, A and B). Three weeks after a single sensitizing dose of either trinitrochlorobenzene (Fig 3, A) or dinitrofluorobenzene (which was similar to trinitrochlorobenzene; data not shown), there was a low-level hapten specific IgE response to these haptens that was detectable only in B7-1 transgenic mice, not in B7-2 or nontransgenic mice. Antihapten IgM and IgG were equivalent in all 3 groups (data not shown).

Repeated elicitions on the pinna of the ear of hapten-sensitized mice have been demonstrated to result in the development of hapten-specific IgE.^{33,34} This technique was exploited to determine whether the low-level IgE could be amplified *in vivo*. Under these circumstances, sera from B7-1 transgenic mice exhibited a robust hapten-specific IgE response compared with the nontransgenic or B7-2 transgenic mice, which exhibited a detectable but low-level hapten-specific IgE response compared with prebleed sera (Fig 3, B).

To confirm the significance of the hapten-specific IgE in B7-1 transgenic mice, groups of B7-1, B7-2, or nontransgenic mice (N = 5) were sensitized with dinitrofluorobenzene on days 0 and +1 and challenged at day +21, with immediate ear swelling responses measured over time (from minutes to as long as 6 hours after challenge). Only in B7-1 transgenic mice was there an immediate ear swelling response to dinitrofluorobenzene challenge (Fig 4, A), which was significantly greater than the ear swelling noted in either the B7-2 or the nontransgenic mice. Histologic study of these immediate reactions revealed mast cell degranulation (data not shown). The significance of the hapten-specific IgE was further

confirmed by passively transferring cutaneous anaphylaxis to naive C57BL/6 mice. Sera from B7-1 transgenic mice, but not B7-2 or nontransgenic mice, induced immediate ear swelling on challenge with topical 0.2% dinitrofluorobenzene (Fig 4, B). This passive transfer was dinitrofluorobenzene-specific, because there was no immediate ear swelling on topical challenge of naive mice with an irrelevant hapten, 0.5% fluorescein isothiocyanate (data not shown). Because both IgE and IgG1 can passively transfer cutaneous anaphylaxis,³⁵ magnetic beads were coated with anti-IgE or anti-IgG1 to adsorb these antibodies and thus define their role in the passive transfer of cutaneous anaphylaxis. Incubation of the sera from B7-1 transgenic mice allergic to dinitrofluorobenzene with magnetic beads coated with anti-IgE or anti-IgG1 before passive transfer indicates that IgE alone was responsible for the immediate ear swelling, because the anti-IgE incubation, but not the IgG1, prevented passive transfer of cutaneous anaphylaxis (Fig 4, C).

Late-phase cutaneous reactions to allergens associated with T_H2 -lymphocyte-mediated immune responses may be associated with infiltration with mast cells.²⁸ Thus, histologic sections of chronic ear swelling responses in dinitrofluorobenzene-sensitized B7-1 transgenic, B7-2 transgenic, and nontransgenic mice were stained with Giemsa. There was a significantly greater number of dermal mast cells present in the chronic inflammatory reactions of B7-1 transgenic mice compared with B7-2 transgenic or nontransgenic mice. Digital image analysis of mast cell numbers of Giemsa-stained ear skin sections (mean number of mast cells/high powered field) was as follows: baseline: nontransgenic, 2.0 ± 1.2 ; B7-1 transgenic, 2.6 ± 1.2 ; B7-2 transgenic, 2.6 ± 1.0 ; 1 week after challenge on the ear: nontransgenic, 3.7 ± 1.2 ; B7-1 transgenic, 14.9 ± 4.7 , $P < .01$ ANOVA compared with nontransgenic or B7-2 transgenic mice, highly significant; B7-2 transgenic, 5.0 ± 1.2).

DISCUSSION

Our transgenic mouse model of CHS suggests that peripheral costimulation by keratinocytes can have significant effects on the immune response to percutaneous haptens. Whereas haptens such as trinitrochlorobenzene and dinitrofluorobenzene normally elicit T_H1 immune responses in C57BL/6 mice,³⁷⁻⁴¹ the presence of hapten-specific IgE only in B7-1 transgenic mice of this background suggests that T_H2 lymphocytes contributed to the immune response. This is novel because it is the first demonstration that epithelial-derived (ie, tissue-based) costimulation can influence the characteristics of the antihapten immune response. Previous studies of the effect of costimulation on T_H2 -lymphocyte development during immune responses have focused on B7-1 and B7-2 associated with professional APCs that migrate to peripheral lymph nodes such as dendritic cells, monocytes, and B lymphocytes.¹⁹⁻²⁴

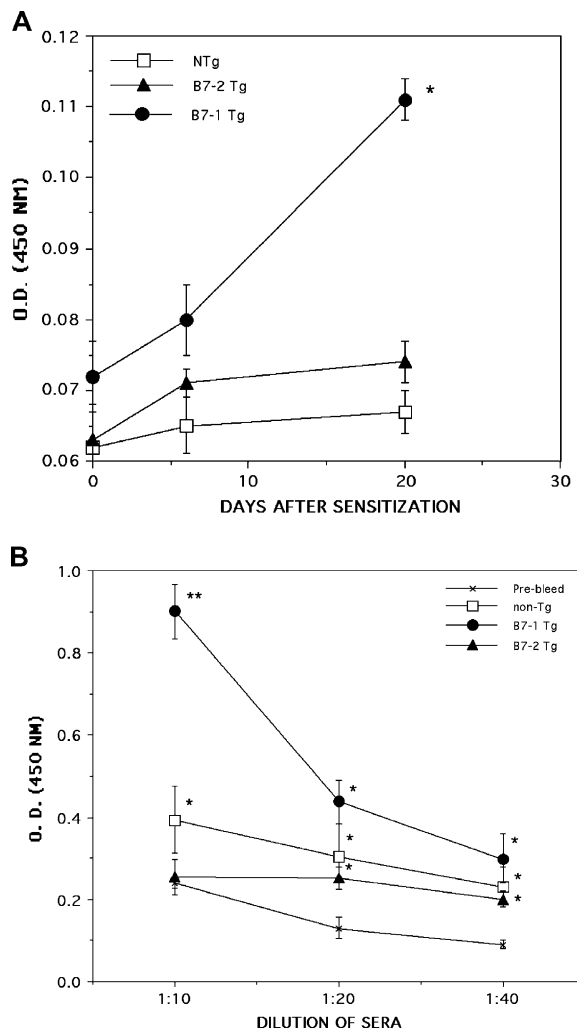
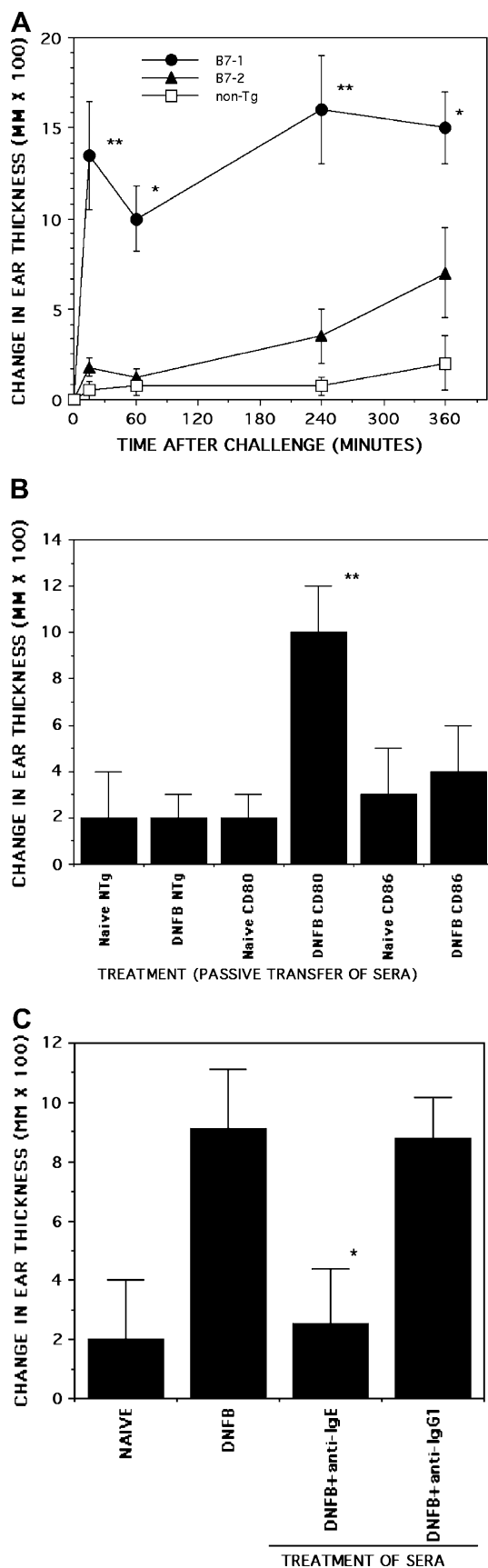


FIG 3. Hapten-specific IgE production in B7-1 transgenic (Tg) mice. Groups of mice ($N = 5$ /group) were sensitized with trinitrochlorobenzene (**A**) or dinitrofluorobenzene (**B**). Sera were obtained at the indicated intervals, and hapten-specific IgE was assayed by using an ELISA. **A**, trinitrophenyl-specific IgE production after trinitrochlorobenzene sensitization; **B**, DNP-specific IgE production 4 weeks after sensitization and repeated elicitation on the pinna of the ear. ($\dagger P < .001$; $*P < .05$ compared with prebleed sera). NTg, Nontransgenic.

The hapten-specific IgE detected in B7-1 transgenic mice was relevant to the pathophysiology of the early component of CHS, because only B7-1 transgenic mice exhibited immediate ear swelling after hapten challenge, and sera from these mice could passively transfer immediate reactivity to naive mice. Furthermore, this model system was developed in C57BL/6 mice, a strain of animals that has a propensity to develop T_H1 -lymphocyte-mediated immune responses against haptens or infectious agents.⁴² Thus, the emergence of a significant hapten-specific IgE response to T_H1 -driven haptens in C57BL/6 mice indicates that B7-1 but not B7-2-mediated costimulation exhibits activity for the development T_H2 lymphocytes.



A similar IgE response that results in T_H2 -lymphocyte emergence and immediate type hypersensitivity has been noted only in models in which sensitized mice are repeatedly elicited with haptens such as dinitrofluorobenzene.^{28,33,34} A weak but detectable hapten-specific IgE response is present after a single sensitizing dose of hapten only in B7-1 transgenic mice, not B7-2 transgenic or nontransgenic mice (Fig 3, A). When hapten-sensitized mice were exposed to repeated hapten elicitations on the pinna of the ear in our experimental system as previously described in the literature,^{28,33,34} the B7-1 transgenic mice developed robust hapten-specific IgE, and the B7-2 transgenic and nontransgenic mice developed weak but detectable IgE responses (Fig 3, B).

The common scenario in both models is the presence of chronic inflammation and T_H2 -promoting cytokines such as IL-10. Both of these models suggest that exaggerated or persistent inflammation plays an important role in the emergence of T_H2 -lymphocyte development to the point that IgE becomes a significant mediator of inflammation.

Previous studies of B7-1 and B7-2 gene-targeted mice have suggested that these molecules play a role in antibody isotype switching in response to hapten application.⁴³ These studies did not address a possible role for keratinocyte-derived B7-1, nor did they address the role of costimulation in hapten-specific IgE responses.

Under normal conditions, human keratinocytes have been found to express B7-1 at a low level^{7,8} insufficient for T-cell activation. B7-2 has not been detected in human keratinocytes. However, in T-cell-dependent inflammatory skin disease states, such as allergic contact dermatitis, psoriasis, and lichen planus, keratinocytes in the affected areas increase their cell surface expression of B7-1, as revealed by immunohistochemical studies.⁷⁻¹² The increased cell surface expression of B7-1 could theoretically play a role in amplifying the T-cell-mediated hypersensitivity reactions seen in these skin conditions. Human keratinocytes exposed to certain allergens and irritants transcriptionally upregulate their expression of the B7-1 gene¹² from 2 to 10 times higher than control keratinocytes in an *in vitro* reporter assay. Thus, this transgenic mouse model may be highly relevant to understanding the pathophysiology of human responses to contact allergens.

Atopic dermatitis is a common dermatologic disorder with a strong genetic component.^{44,45} Several candidate genes have been proposed, including 1 study that suggested linkage to chromosome 3q21, a region that encodes the costimulatory molecules B7-1 and B7-2.⁴⁶ Our data

FIG 4. Immediate ear swelling and passive transfer of cutaneous anaphylaxis of sera from B7-1 transgenic (Tg) mice, B7-2 Tg mice, or nontransgenic (NTg) mice. Groups of mice (N = 5) were sensitized with 0.5% dinitrofluorobenzene (DNFB) as described in Methods. **A**, After challenge, ear swelling was measured. **B**, Passive transfer of cutaneous anaphylaxis from B7-1 Tg mice allergic to DNFB to naive C57BL/6 mice ($\dagger P < .01$, ANOVA, compared with control sera). **C**, Anti-IgE-magnetic but not anti-IgG1-magnetic beads block passive transfer of cutaneous anaphylaxis ($*P < .05$, ANOVA, compared with untreated serum from B7-1 Tg mice allergic to DNFB).

further suggest a novel role for the costimulatory molecules on epithelial cells in the pathogenesis of aberrant T_H2-lymphocyte-mediated immune responses.

The relevance of our mouse model system to the mechanisms of human CHS remains to be determined. It is noteworthy that many patients who have developed allergic contact dermatitis to xenobiotics frequently exhibit chronic inflammation in the skin as a result of multiple eliciting exposures to the allergens over time. Thus, our B7-1 transgenic mouse model, when combined with the existing multiple exposure method, may be relevant to the skewing of the immune response toward T_H2, with a possible contribution by keratinocyte-derived costimulation.

Studies of B7-1 expression and function on human keratinocytes from patients with atopic dermatitis compared with those of normal, nonatopic controls will elucidate the role of keratinocyte-derived costimulation in this allergic skin disorder.

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