α4β7 Integrin is essential for contact hypersensitivity by regulating migration of T cells to skin

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Background: β7 Integrin, a cell adhesion molecule, is present in the form of α4β7 integrin or αEβ7 integrin. α4β7 Integrin is expressed on most leucocytes and is essential for their migration to gut-associated lymphoid tissues by interacting with its primary ligand, mucosal addressin cell adhesion molecule-1, which is preferentially expressed in gut-associated lymphoid tissues. Although the importance of α4β7 integrin in intestinal inflammation has been established, its role in cutaneous inflammation remains to be elucidated.

Objective: We sought to investigate the role of β7 integrin in cutaneous inflammation.

Methods: We used a murine contact hypersensitivity model and examined the role of β7 integrin by using β7 integrin–deficient and αE integrin–deficient mice.

Results: β7 Integrin–deficient mice, not αE integrin–deficient mice, are defective in contact hypersensitivity responses. β7 Integrin deficiency does not affect irritant contact dermatitis. The distribution, migration, and function of antigen presenting cells from β7 integrin–deficient mice are comparable to those from wild-type mice. Moreover, sensitized β7 integrin–deficient T cells are able to respond to antigen stimuli *in vitro* and elicit contact hypersensitivity responses when directly injected into the skin. However, they are defective in reaching the skin under inflammatory conditions, resulting in reduced contact hypersensitivity responses when intravenously injected. Furthermore, intraperitoneal injection of anti–α4β7 integrin neutralizing antibody elicit impaired contact hypersensitivity responses.

Conclusion: α4β7 Integrin contributes to contact hypersensitivity responses by regulating T-cell migration to inflammatory skin. (J Allergy Clin Immunol 2010;126:1267-76.)

Key words: *α4β7 Integrin, contact hypersensitivity responses, VCAM-1*

Abbreviations used

$\alpha E^{-/-}$:	αE Integrin–deficient
APC:	Antigen presenting cell
$\beta 7^{-/-}$:	β7 Integrin–deficient
CFSE:	Carboxyfluorescein diacetate succinimidyl ester
CHS:	Contact hypersensitivity
CLA:	Cutaneous lymphocyte-associated antigen
DC:	Dendritic cell
DLN:	Draining lymph node
DNBS:	2,4-Dinitrobenzene sulfonic acid
DNFB:	2,4-Dinitrofluorobenzene
FACS:	Fluorescence-activated cell sorting
FITC:	Fluorescein isothiocyanate
MAdCAM-1:	Mucosal addressin cell adhesion molecule-1
oxazolone:	2-Phenyl-4-ethoxymethylene oxazolone
VCAM-1:	Vascular cell adhesion molecule-1
WT:	Wild-type

Leukocytes recirculate through blood and secondary lymphoid organs, and they also migrate into peripheral tissues to elicit immune responses.[1] Aberrant leukocyte trafficking, however, leads to inflammatory and autoimmune diseases. Leukocyte trafficking is orchestrated and controlled by combinatorial inputs of adhesion and chemoattractant molecules located on both leukocytes and vascular endothelium.

Integrins, a family of cell adhesion molecules, regulate leukocyte adhesion and migration into blood vessels of lymphoid organs and at sites of inflammation. Integrins are composed of an α subunit and a β subunit. β7 Integrin forms heterodimer either with α4 integrin or αE integrin.[2] α4β7 Integrin is expressed on lymphocytes,[3] macrophages,[4] mast cells,[5] natural killer cells,[6] and eosinophils,[7] and its ligands includ mucosal addressin cell adhesion molecule-1 (MAdCAM-1)[8,9] and vascular cell adhesion molecule-1 (VCAM-1).[10] MAdCAM-1 is the main ligand of α4β7 integrin and is expressed on Peyer's patches of intestines, high endothelium venules of mesenteric lymph nodes, and vascular endothelial cells of gut lamina propria.[11,12] VCAM-1 expressed on activated vascular endothelial cells is induced by inflammation.[10] VCAM-1 binds to α4β1 integrin in addition to α4β7 integrin.[13]

α4β7 Integrin organizes migration of leukocytes to Peyer's patches and mesenteric lymph nodes by binding to MAdCAM-1. Involvement of β7 integrin in intestinal immunity has been extensively explored. In murine models, migration of activated $CD8^+$ T cells to the mesenteric lymph nodes and Peyer's patches after viral infection depends on α4β7 integrin.[14] α4β7 Integrin is also important for the invasion of alloreactive donor T cells into the gut and the subsequent development of intestinal graft-versus-host disease.[15] In another study, pathogenic $CD4^+$ T cells use the α4β7 integrin/MAdCAM-1 pathway to recirculate to the

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chronically inflammatory small intestine in models of Crohn disease–like ileitis.[16] In human beings, MLN0002, a mAb targeting the α4β7 integrin, is effective for disease control against both ulcerative colitis[17] and Crohn disease.[18] As for αEβ7 integrin, the onset and maintenance of murine Crohn disease models depend on the colonic localization of αEβ7 integrin-expressing lamina propria $CD4^+$ T cells.[19]

On the other hand, little has been known about the engagements of β7 integrin in cutaneous inflammation. β7 Integrin–deficient ($\beta7^{-/-}$) mice show delayed skin graft rejections,[20] and αE integrin–deficient ($\alpha E^{-/-}$) mice, in certain backgrounds, are known to develop dermatitis.[21] However, the mechanisms of these findings were far from clear. It is well known that VCAM-1, one of the ligands for α4β7 integrin, is upregulated at sites of cutaneous inflammation.[10] Therefore, we set out to examine the role of β7 integrin in cutaneous immune responses by using contact hypersensitivity (CHS) models. The results of this study indicate that α4β7 integrin contributes to CHS responses by regulating T-cell migration to inflammatory skin.

METHODS

Mice

C57BL/6 mice were purchased from SLC Japan (Tokyo, Japan). $\beta7^{-/-}$ mice were generated as described.[22] $\alpha E^{-/-}$ mice were obtained from Jackson Laboratory (Bar Harbor, Me). All mice were free of pathogenic bacteria and viruses as determined by antibody screening and routine histologic analysis of organs and tissues. All experiments were performed by using mice between 8 and 14 weeks of age. All studies and procedures were approved by the Animal Experiment Committee of the Graduate School of Medicine of the University of Tokyo guided by the Bioscience Committee of the University of Tokyo.

CHS responses

Contact hypersensitivity responses were induced by using 2,4-dinitrofluorobenzene (DNFB) as previously described.[23] The volume 50 μL 0.5% DNFB in 4:1 acetone/olive oil solution was painted on the shaved abdomens of mice on days 0 and 1. Mice were challenged on day 6 by topically applying 20 μL 0.25% DNFB (10 μL/side of the pinna) onto each side of the left ear. The right ear received the vehicle alone. CHS responses to 2-phenyl-4-ethoxymethylene oxazolone (oxazolone) were induced by applying 150 μL 3% oxazolone in 3:1 alcohol/acetone solution on the shaved abdomens on day 0, and then challenged on day 7 with 20 μL 1% oxazolone (10 μL/side). CHS responses to fluorescein isothiocyanate (FITC) were induced by applying 400 μL 0.5% FITC in 1:1 acetone/dibutyl phthalate to the shaved abdomen. Six days later, the CHS reactions were elicited by applying 20 μL of the FITC solution (10 μL/side). For all CHS experiments, baseline ear thickness was determined with a caliper (dial thickness gauge; Ozaki Manufacturing, Tokyo, Japan). Ear swelling responses were measured at 24 hours after elicitation, and the change in ear thickness from baseline measurement was computed. Each ear was measured 5 times, and the mean of these values was used. Croton oil was used to elicit irritant contact dermatitis: 15 μL 1.0% croton oil in 4:1 acetone/olive oil solution was painted on the pinna, and 16 hours later, the change in ear thickness from baseline was measured as described for CHS responses. DNFB, oxazolone, FITC, and croton oil were purchased from Sigma-Aldrich (St Louis, Mo).

Histologic examination

Ears were excised 24 hours after elicitation with DNFB. Excised ears were fixed in 2% paraformaldehyde and embedded. Sections, 4 μm thick, were stained with hematoxylin and eosin. For immunohistochemistry, epidermal sheets were stained with phycoerythrin-conjugated anti–$I\text{-}A^b$ antibody (AF6-120.1; BD Pharmingen, San Diego, Calif).

Purification of cell subsets

Lymph nodes (inguinal and axillary) and spleens were used to prepare single-cell suspensions. T cells were isolated by negative selection by using the magnetic-activated cell sorting system (pan T-cell isolation kit; Miltenyi Biotec, Bergisch Gladbach, Germany). Purity was routinely more than 95%. For obtaining dendritic cells (DCs) from skin, mouse ear skin was placed onto the 6-well plates containing RPMI 1640 medium supplemented with glutamine, antibiotics, and 10% FBS (1.5 mL/well) and incubated for 5 days at 37°C in 5% CO_2. DCs from skin draining lymph nodes (DLNs) were prepared by enzymatic digestion with 1 mg/mL collagenase D (Sigma-Aldrich) and 0.2 mg/mL deoxyribonuclease (Sigma-Aldrich). After digestion, cell suspension was layered on 14.5% metrizamide (Accurate Chemical & Scientific Corp, Westburg, NY) and centrifuged. By discarding the top layer of the suspension, DCs were collected at the interface.

Antigen-specific proliferation assay

In vitro proliferation to 2,4-dinitrobenzene sulfonic acid (DNBS; Sigma-Aldrich), the water-soluble analog of DNFB, was carried out as follows. In all experiments, cells were cultured in the presence of DNBS for 3 days. In some experiments, inguinal and axillary lymph node cells collected on day 1 after sensitization to DNFB were used as antigen presenting cells (APCs). Inguinal and axillary lymph node cells from unsensitized mice were also used as APCs, and they were incubated for 20 minutes at 37°C with 4 mmol/L DNBS before use. APCs (5×10^5) were then treated with 25 μg/mL mitomycin C (Calbiochem, Darmstadt, Germany) for 25 minutes and incubated for 3 days with purified T cells harvested on day 5 after DNFB sensitization. Cultures were pulsed with 1 μCi/well [^{3}H] thymidine (Amersham Bioscience, Buckinghamshire, United Kingdom). T-cell proliferation was determined in triplicate wells of 96-well plates by [^{3}H] thymidine uptake during the last 18 hours. All experiments were performed at least 3 times.

Ex vivo chemotaxis assay

2,4-Dinitrofluorobenzene–painted mouse ear skin was placed onto 6-well plates containing RPMI 1640 medium supplemented with glutamine, antibiotics, and 10% FBS (2 mL/well) and incubated for 24 hours at 37°C in 5% CO_2. Migratory DCs were collected, stained with propidium iodide (BD Pharmingen), and FITC-conjugated CD11c antibody (HL3; BD Pharmingen). The proportion of $CD11c^+$ cells per propidium iodide–negative population was calculated by using a FACScan flow cytometer (Becton Dickinson, Franklin Lakes, NJ) and CellQuest software (Becton Dickinson).[24]

Adoptive transfer of sensitized lymph node cells

Donor mice were sensitized with DNFB as described. On day 6, T cells were isolated from DLNs by negative selection using a pan T-cell isolation kit (Miltenyi Biotec). Naive recipient mice intravenously received 5×10^5 T cells, which were from donor mice, in 200 μL PBS. Eighteen hours later, mice were elicited with 0.25% DNFB, and after 24 hours, ear swelling responses were measured. As a control, unsensitized wild-type (WT) or $\beta7^{-/-}$ mice were painted with 0.25% DNFB on the pinna. In another experiment, sensitized 2×10^6 T cells in 20 μL PBS were intradermally injected into both ears using a 27-gauge needle. Recipients were immediately treated with 0.25% DNFB solution on the left ears and vehicle on the right ears. Ear swellings were measured after 24 hours.

T-cell recruitment to CHS sites

The procedure was performed as previously described with some modification.[25] DNFB-primed T cells were prepared as described and labeled with 1 μmol/L carboxyfluorescein diacetate succinimidyl ester 5-chloromethylfluorescein diacetate (CFSE; Molecular Probes, Eugene, Ore). Naive recipient mice intravenously received 5×10^6 labeled T cells and were challenged with