Circulating Allergen-reactive T Cells from Patients with Atopic Dermatitis and Allergic Contact Dermatitis Express the Skin-selective Homing Receptor, the Cutaneous Lymphocyte–associated Antigen

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Summary

The cutaneous lymphocyte–associated antigen (CLA) is the major T cell ligand for the vascular adhesion molecule E-selectin, and it has been proposed to be involved in the selective targeting of memory T cells reactive with skin-associated Ag to cutaneous inflammatory sites. To further investigate the relation of CLA and cutaneous T cell responses, we analyzed the CLA phenotype of circulating memory T cells in patients with allergic contact dermatitis and atopic dermatitis (AD) alone vs in patients manifesting bronchopulmonary atopy (asthma with or without AD) and nonallergic individuals. Significant T cell proliferative responses to Ni, a contact allergen, and to the house dust mite (HDM), an allergen to which sensitization is often observed in AD and/or asthma, was noted only in allergic and atopic individuals, respectively. When the minor circulating CLA+CD3+CD45RO+ subset was separated from the major CLA−CD3+CD45RO+ subpopulation in Ni-sensitive subjects, the Ni-dependent memory T cell response was largely confined to the CLA+ subset. A similar restriction of the T cell proliferative response to the CLA+ memory subset was observed for HDM in patients with AD alone. In HDM-sensitive patients with asthma with or without AD, however, the CLA− subset exhibited a strong antigen-dependent proliferation, in contrast to patients with AD alone, whose CLA− subset proliferated very weakly to HDM. In asthma with or without AD, the HDM-dependent proliferation slightly predominated in the CLA− when compared to the CLA+ subset. The functional linkage between CLA expression and disease-associated T cell effector function in AD was also demonstrated by the finding that the circulating CLA+ T cell subset in AD patients, but not nonatopic controls, selectively showed both evidence of prior activation (human histocompatibility antigen–DR expression) and spontaneous production of interleukin 4 but not interferon-γ. Taken together, these observations demonstrate the correlation of CLA expression on circulating memory T cells and disease-associated memory T cell responses in cutaneous hypersensitivity, and they suggest the existence of mechanisms capable of sorting particular T cell Ag specificities and lymphokine patterns into homing receptor–defined memory subsets.
process, and we and others have proposed that tissue-selective T cell extravasation in the skin is mediated, at least in part, by the interaction between the cutaneous lymphocyte-associated antigen (CLA) on skin-homing T cells and its vascular counterreceptor, E-selectin, on inflamed superficial dermal postcapillary venules (10–17). In keeping with this hypothesis, CLA is expressed by a minor subset of CD45RO+ memory T cells in peripheral blood, by essentially all such cells in cutaneous sites of chronic inflammation, but by relatively few T cells in extracutaneous inflammatory sites (10, 14). Moreover, CLA is expressed by the malignant T cells of chronic-phase cutaneous T cell lymphoma (mycosis fungoides and Sézary syndrome), but not by non–skin-associated T cell lymphomas/leukemias (10, 15). CLA expression is regulated at the time of T cell activation by microenvironmental factors (including cytokines such as TGF-β1 and IL-12) and has been demonstrated to be selectively induced on T cells undergoing the virgin to memory transition in skin-draining peripheral lymph nodes (PLN) (16 and Picker, L. J., unpublished data).

Since CLA is selectively associated with infiltrating T cells of the skin, it is possible that a similar association of CLA and skin-associated T cell functions exists in the blood. To investigate this possibility, we compared the distribution of disease-associated memory T cells in two skin-associated and MHC-restricted (18–20) immune responses to well-characterized Ags—allergic contact dermatitis (CD) to Ni and atopic dermatitis (AD) associated with hypersensitivity to the house dust mite (HDM)—to that of T cells specific for tetanus toxoid (TT) in normal individuals, and to disease-associated T cells in patients who manifest bronchopulmonary atopy (asthma). Our results demonstrate the association of CLA expression on circulating memory T cells and cutaneous immune function in AD and CD, and they provide a model for the study of the mechanisms responsible for the selective distribution of the memory T cell repertoire among the different homing marker–defined T cell subsets.

Materials and Methods

Study Population. 14 patients with AD alone (mean age: 36 yr), 4 patients with AD plus asthma (mean age: 36 yr), 3 patients with asthma alone (mean age: 36 yr), 9 patients with Ni contact allergy (mean age: 35 yr), and 7 healthy controls (mean age: 36 yr) were analyzed in this study. All patients with AD fulfilled the criteria of Hanifin and Rajka (21). Atopics were skin prick test positive for HDM and presented HDM-specific IgE in serum. Ni-allergic CD patients showed positive epicutaneous patch test reactions to the house dust mite (HDM) allergen (DerP1) (10, 11, 14, and IFN-γ Assays. mAbs mAbs anti-CD45RA were purchased from Immunotech. Anti-CD14, anti-CD19, and streptavidin-conjugated magnetic microbeads were obtained from Miltenyi Biotech (Bergisch Gladbach, Germany).

HDM (Dermatophagoides pteronyssinus) extract was obtained from Haarlems Allergenen Laboratorium (Haarlem, The Netherlands). Purified Dermatophagoides pteronyssinus allergen (DerPi) was from Dr. H. Löwenstein (ALK, Horsholm, Denmark). Purified TT was obtained from the Swiss Serum and Vaccination Institute (Bern, Switzerland).

Preparation of CLA-enriched and CLA-depleted Memory T Cells. PBMC were isolated by Ficoll density gradient (Biochrom K.G., Berlin, Germany) centrifugation and resuspended in DMEM (Gibco, Basel, Switzerland) supplemented with 10 mM Hepes (Gibco), 5 mM EDTA (Fluka Chemie AG, Buchs, Switzerland), 2% FCS (Ser a-Lab, Crawley Down, UK), 100 U/ml penicillin (Gibco), and 100 µg/ml streptomycin (Gibco). Cells were resuspended in anti-CD4 and anti-CD19 conjugated to magnetic microbeads (Miltenyi Biotech) and were washed through the magnetic activated cell separation system (MACS; Miltenyi Biotech) to obtain autologous APC. The CD14- and C19-depleted cells were incubated with anti-CD45RA and anti-CD16 for 30 min on ice. Washed cells were mixed together with goat anti–mouse–conjugated magnetic microbeads to negatively select a population of CD45RO+ T cells. CD45RO+ T cells were incubated with anti-CLA HECA-452 mAb for 30 min on ice. Washed cells were then incubated with biotin-conjugated goat anti–rat (H+L) for 30 min on ice. Finally, washed cells were mixed with streptavidin conjugated to magnetic microbeads and were washed through the MACS. Both nonmagnetic (CLA−) and magnetic (CLA+) cells were recovered by sequential elution.

Proliferation Assays. The CLA-enriched and memory T cells (5 × 10⁴ cells per well) were incubated with 700 R irradiated autologous APC (10⁴ cells per well) and with or without HDM (30 µg/ml), DerPi (10 µg/ml), or NiSO₄ (20 µg/ml) in 250 µl of RPMI 1640 containing 10% complement-inactivated autologous serum, 2 mM L-glutamine (Gibco), 100 U/ml penicillin, and 100 µg/ml streptomycin in 96-well U-bottom plates (Falcon Labware, Becton Dickinson and Co., Cockeyesville, MD). The cultures were pulsed with 1 µCi of [3H]thymidine (DuPont/New England Nuclear, Boston, MA) after 5 d, and the incorporation of the labeled nucleotide was determined after 16 h.

Cell Staining and Flow Cytometry. Five-parameter analysis was performed on an Epics Profile (Coulter Corp.) installed with a power pack and equipped with a 488-nm argon laser. Fluorescence 1 was measured using a 525-nm band pass filter; fluorescence 2 was measured with a band pass filter of 635 nm. Typically, 10⁵ cells were recovered by sequential elution. As delayed hypersensitivity to Ni manifesting as CD is exclusively expressed in the skin, we investigated the in vitro T cell response confined to
proliferative response of CLA+ and CLA− T cells to this allergen that is recognized in the context of MHC class II molecules (19, 23). Significant T cell proliferation to Ni was only observed in individuals with a positive patch test to Ni (data not shown). In these sensitive individuals (mean: 10.1% CLA+ in CD3+ PBMC; range: 7.0–18.3%), the proliferative response to the allergen was exclusively confined to the CLA+ circulating memory T cell subset in seven of nine subjects and nearly exclusively confined to this subset in the other two subjects (Fig. 1 A; p = 0.0025 for the comparison of CLA+ and − subsets for the overall group of nine subjects). In contrast, the proliferative response to TT was not confined to CLA+ memory T cells in five healthy subjects (mean: 12.0% CLA− in CD3+ PBMC; range: 7.1–20.3%) and in four Ni-sensitive individuals (Fig. 1 B), demonstrating that not all antigen-induced memory T cell responses consistently predominate in the CLA+ subset.

AD is another skin-based T cell–mediated chronic hypersensitivity disorder in which responsible allergens can sometimes be identified. Unlike CD to Ni, however, atopic individuals may manifest hypersensitivity responses to a particular allergen in skin alone (AD), in an extracutaneous tissue alone (primarily the lung, as in asthma), or in multiple tissues (either simultaneously in skin and lung or often at different times). The physiologic factors responsible for determining these tissue “preferences” of disease expression are unknown, but they may reflect differences in the regulation of homing receptor expression on developing memory T cells. To test this hypothesis, we identified a group of atopic individuals with demonstrable hypersensitivity and MHC-restricted (18, 20, 24–26) T cell reactivity to HDM who manifested atopic symptoms in the skin alone (n = 7) or in the lung alone or with skin (n = 7). As shown in Fig. 2 A, memory T cell proliferative responses to HDM and DerP1 were preferentially detected in CLA+ T cells in six out of seven patients with AD alone (p = 0.006; mean: 7.3% CLA+ in CD3+ PBMC; range: 2.5–11.6%). In contrast, in those individuals manifesting asthma alone (mean: 8.2% CLA+ in CD3+ PBMC; range: 3.4–13.0%) or asthma with AD (mean: 14.3% CLA+ in CD3+ PBMC; range: 2.9–22.4%), the CLA− subset exhibited a stronger HDM-dependent proliferative response than the CLA+ subset in patients with AD alone (p = 0.0045; Fig. 2). In all the patients with asthma...
with or without AD, there was a slightly higher proliferative response to HDM in the CLA- subset.

To further investigate the linkage between disease-associated T cells in AD and CLA expression, we asked whether patients with active AD may have increased numbers of circulating activated T cells, and if so, whether these cells expressed CLA. As shown in Fig. 3 A, patients with active AD did indeed demonstrate a significantly higher number of cells expressing the activation marker HLA-DR within the circulating CLA+ T cell subset as compared to the CLA- subset (mean: 6.18 ± 1.6%, 1 SEM, vs. 1.56 ± 0.6, p = 0.0035). This is in contrast to the situation in normal individuals, who demonstrate much lower overall HLA-DR expression and no preferential expression of the CLA+ T cell subset (Fig. 3 B). Moreover, in AD subjects (Fig. 3 C), unlike in normal control subjects (data not shown), a high level of spontaneous IL-4 production was observed after in vitro incubation of the CLA+, but not CLA-, memory T cell subset. Furthermore, IFN-γ was not detected in these subjects in either T cell subset (data not shown). Given that IL-4-producing (Th2-type) T cells have been strongly implicated in the pathogenesis of AD (27-31), these results suggest that when dermatitis is the primary presentation of atopy, disease-associated effector T cells are primarily in the CLA+ memory subset.

Discussion

AD and allergic CD are two independent forms of cutaneous allergy that are associated with an allergen-dependent T cell response (32). In animal models, it has clearly been documented that allergic CD is T cell dependent and that the allergen-specific T cells migrate into the site of allergen application (33). There they may induce the clinical features of dermatitis, which include swelling, erythema, and secondary infiltration, first by granulocytes and later by mononuclear cells. There is evidence that allergic CD is a type 1 Th response (34). Although it has not formally been shown that type 2 Th are responsible for AD, type 2 Th clones with specificity for atopy-related Ag's can be derived from the skin of patients with AD (27-29). In addition, so-called late-phase cutaneous reactions in atopics contain many T cells and exhibit positive signals for type 2 Th lymphokines by in situ hybridization (30). Although there is presently no animal model for AD, activated type 2 Th injected into the skin can induce inflammation that is dependent on IL-4 (31). Similarly, type 2 Th cytokines are found in bronchoalveolar cells from patients with allergic asthma (35, 36). Such IL-4- and IL-5-producing T cells in the bronchial wall may be responsible for the recruitment of eosinophils and other proinflammatory cells, as well as for the late inflammatory phases of asthma.

In addition to the skin-selective expression of CLA in organ-infiltrating T cells, evidence linking CLA expression of memory T cells to skin-selective homing includes (a) the pattern of expression of other tissue-selective homing receptors on the CLA+ T subset (high L-selectin; low α4β1- and αεβ7-integrins; 14); (b) the high expression of CLA on allergen-specific T cell clones from an AD patient vs the lack of CLA on non-Ag-specific blood-derived clones from the same patient (13); and (c) the selective up-regulation of CLA on T cells undergoing the virgin to memory transition in skin-draining PLNs vs mucosal secondary lymphoid tissues (16). Here we provide further evidence for the association of CLA and skin immune responses by demonstrating that the circulating Ag-specific memory T cells in subjects with skin-restricted chronic inflammation conditions are highly restricted to the CLA+ subset. Moreover, in AD patients, circulating HLA-DR-expressing and “spontaneously” IL-4-producing T cells were selectively enriched in the CLA+ memory subset.
subset. In contrast, the CLA− subset was the major source of HDM reactivity in HDM-sensitive asthmatic individuals. Taken together, our observations are compatible with the skin-homing function of CLA, and they suggest the existence of mechanisms that are capable of selectively targeting memory T cells with a particular Ag reactivity and lymphokine pattern into a distinct homing marker–defined subset.

We have previously proposed that homing receptor expression, like effector functions such as cytokine synthesis potential (37), is regulated at the time of T cell activation, initially during the virgin to memory transition in secondary lymphoid tissue, and perhaps also at subsequent "reactivations" during the virgin to memory transition in secondary lymphoid tissue, and perhaps also at subsequent "reactivations" (16). However, similar to what has been described for cytokine production, the specific regulatory elements directing homing function in vivo are likely to be complex and thus may not always correlate with anatomic site of antigen deposition alone. Nevertheless, this hypothesis would predict that T cells reactive with an Ag that consistently is encountered by the immune system in CLA-inducing microenvironment would, over time, preferentially accumulate in the skin-homing CLAhigh, L-selectin+, α4β1-integrin− memory T cell subset.

Contact allergens such as Ni would clearly fit into the category of tissue (e.g., skin)-associated Ag's, and our finding that Ni-reactive memory T cells are almost exclusively in the CLA− subset supports this hypothesis. It is much less clear how Ag's such as HDM interact with the immune system, and indeed the varied organs of atopy manifestations in different patients and in the same patient at different times suggest variability of the microenvironment in which specific T cells of atopics encounter HDM Ag. Clearly, however, in some individuals with atopic manifestations restricted to the skin, HDM-specific T cells are preferentially in the CLA− memory subset. It remains to be determined in these patients whether this preferential CLA induction on HDM-reactive memory T cells results from HDM preferentially entering the body via cutaneous routes or from genetic or other regulatory influences promoting CLA induction in non–skin-associated microenvironments.

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