

Phenotypic characterization of T cells infiltrating the conjunctiva in chronic allergic eye disease

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Background: Chronic allergic conjunctivitis comprises a spectrum of diseases including atopic keratoconjunctivitis, atopic blepharoconjunctivitis, vernal keratoconjunctivitis, and contact lens-associated giant papillary conjunctivitis. Each condition is characterized by a complex immunopathology with a mixed cellular infiltrate. Treatment with conventional mast cell stabilizers is often unsatisfactory, and therapy depends heavily on topical corticosteroids.

Objective: Recent evidence suggests that T lymphocytes play an important role in mediating the immunopathology seen in the chronic allergic response in the skin and the lungs.

Methods: We have therefore investigated the prevalence of T cells and their subsets in the different chronic allergic eye conditions by means of immunohistochemistry.

Results: We found significantly increased numbers of CD4⁺, CD45RO⁺, and HLA-DR⁺ T cells in the conjunctiva of patients with atopic and vernal keratoconjunctivitis and giant papillary conjunctivitis, with a corresponding upregulation of markers present on antigen presenting cells.

Conclusions: These findings suggest that like allergic conditions in the skin and lungs, CD4⁺ memory T cells are involved in the regulation of the immunopathology of chronic allergic eye responses. Further characterization of these T cells will provide the information necessary for future immunotherapeutic interventions. (*J Allergy Clin Immunol* 1996;98:686-96.)

Key words: Allergy, eye, conjunctiva, immunology, T cells, immunohistochemistry

Allergic conjunctivitis comprises a very heterogeneous spectrum of disorders. Based largely on clinical criteria, allergic conjunctivitis includes the rather mild but non-sight-threatening seasonal allergic conjunctivitis and perennial allergic conjunctivitis on the one hand and the severe and potentially sight-threatening chronic vernal keratoconjunctivitis (VKC) and atopic keratoconjunctivitis (AKC) on the other.

Seasonal allergic conjunctivitis, or hay fever conjunctivitis, is the most commonly encountered ocular atopy and is found in association with

Abbreviations used

ABC:	Atopic blepharoconjunctivitis
AKC:	Atopic keratoconjunctivitis
APCs:	Antigen presenting cells
GPC:	Giant papillary conjunctivitis
LC:	Langerhans cell
mAb:	Monoclonal antibody
MLA:	Mucosal lymphocyte antigen
TBS:	Tris-buffered saline
VKC:	Vernal keratoconjunctivitis

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Supported by Fight for Sight, Institute of Ophthalmology, London.

Received for publication Jan. 26, 1996; revised Feb. 13, 1996; accepted for publication Feb. 15, 1996.

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0091-6749/96 \$5.00 + 0 1/1/73119

pollinosis.¹ It is prototypical of an immediate or type I hypersensitivity reaction, involving IgE-dependent release of mast cell mediators. It usually presents relatively mild symptoms and responds well to treatment with H₁-antihistamines and mast cell stabilizers such as sodium cromoglycate.² AKC and VKC display more severe and chronic symptoms and can involve the cornea. In contrast to seasonal allergic conjunctivitis, AKC and VKC

exhibit a more complex immunopathology involving many different inflammatory cells.²

Clinically, AKC is a bilateral chronic ocular manifestation of a systemic hypersensitivity syndrome associated with atopic dermatitis.² The clinical symptoms and manifestations have been described in detail elsewhere.^{2,3} Briefly, patients are first seen with a chronic conjunctivitis, often with papillary hypertrophy of the inferior tarsal conjunctiva and conjunctival scarring. The tissue is hyperemic and edematous, infiltrated by lymphocytes, plasma cells, mast cells, and eosinophils.⁴ Corneal complications may vary in severity from punctate keratitis to frank necrosis. The mechanisms of damage to the cornea in AKC are unclear.⁵

A subgroup of AKC that does not involve corneal complications is referred to as atopic blepharoconjunctivitis (ABC).³

VKC is a bilateral seasonally recurrent condition of the eye in the United Kingdom, which predominantly affects children and is usually self-limiting with a duration of 2 to 10 years.⁶ Like AKC, it can be a painful, debilitating disease, which if left untreated, has sight-threatening consequences as a result of keratopathy.

A spectrum of symptoms and clinical appearance similar to VKC occurs in patients who wear hard and soft contact lenses,⁷ and this condition is referred to as giant papillary conjunctivitis (GPC).⁸ It is generally less serious because there is no corneal involvement, and it occurs in part as a response to denatured proteins that accumulate on the surface of the eye as a result of poor lens hygiene.

Regardless of whether an immediate hypersensitivity reaction is involved in these chronic eye disorders, non-IgE-mediated immune responses undoubtedly contribute to the immunopathology. A mixed cellular infiltrate composed of lymphocytes, plasma cells, mast cells, eosinophils, and in some cases basophils has been described for all these conditions.^{4,7,9} Furthermore, treatment of these chronic variants of allergic eye disease with topical antihistamines and mast cell stabilizers is often unsatisfactory, although almost all respond to corticosteroids.^{6,10} Finally, in VKC and GPC there is not always a clear-cut correlation between allergen-specific IgE responses and the nature and severity of the allergic symptoms.¹¹ It is likely that a cell-mediated immune response is involved in the immunopathology of these disorders, similar to the situation in asthma and atopic eczema.¹²⁻¹⁶ Allergen-specific CD4⁺ T-cell clones, isolated from

atopic individuals, have been shown to secrete lymphokines similar to mouse T_{H2} helper T cells.¹⁷⁻²⁰ These include IL-4, IL-5, IL-6, IL-9, and IL-10.

To establish the degree of involvement of lymphocytes in chronic allergic eye disorders, we examined the cellular infiltrate present in the various chronic allergic eye conditions by using immunohistochemical staining methods. We aimed to look for both similarities and differences between the clinically distinct disease types. In addition, we have assessed the distribution of the T-cell infiltrate within the conjunctiva, the T cells' activation status, and the associated infiltration of monocytes/macrophages, which are also known to be of importance in chronic allergic tissue responses.

METHODS

Subjects and conjunctival biopsies

Subjects recruited for the study included 15 patients with AKC (mean age, 34 years), nine with ABC (mean age, 46 years), 15 with VKC (mean age, 19 years), and 12 with GPC (mean age, 35 years). All patients had active disease requiring treatment, but none had received topical steroids for 3 months or cromoglycate for 1 week before conjunctival biopsy extraction. The biopsy specimens were obtained, after topical lidocaine anesthesia had been induced, from the central third of the superior tarsal conjunctiva with a 3 mm standard trephine. An additional biopsy specimen was obtained from the superior bulbar conjunctiva with scissors.

Control tarsal and bulbar conjunctival specimens were collected from 16 nonatopic age-matched individuals (mean age, 26 years) undergoing squint or cataract surgery under general anesthesia. These patients had received 2 to 4 drops of chloramphenicol 0.5% as preoperative prophylaxis, and specimens were obtained as previously described before the commencement of surgery. All subjects gave informed consent, and the study had ethical committee approval.

Immunohistochemistry

Specimens were fixed immediately in ice-cold acetone containing 2 mmol/L phenylmethylsulfonylfluoride and 20 mmol/L iodoacetamide and were stored at -20°C overnight to prevent protein digestion by endogenous proteases within the tissues during fixation. The biopsy specimens were then post-fixed in acetone and methylbenzoate at room temperature for 15 minutes each and then immersed in a solution of glycolmethacrylate monomer (JB4 embedding kit; Polyscience SA, Warrington, U.K.) and 5% methylbenzoate for 6 hours at 4°C before embedding in the glycolmethacrylate-resin (JB4 embedding kit).²¹ Embedded tissues were stored at -20°C until they were cut for immunohistochemistry.

Sections of 2.0 mm thickness were cut on an ultramicrotome (Reichert-Jung Ultracut E; Leica UK, Ltd.,

TABLE I. Monoclonal antibodies used in the protocol

mAb	Reactivity	Clone	Purchase Supplier	Dilution
CD3	Pan T-cell marker	T3-4B5	DAKO Ltd.	1:100
CD4	T-cell subset (mainly "helper/ inducer" T cells)	MT310	DAKO Ltd.	1:50
CD8	T-cell subset (mainly "suppres- sor/cytotoxic" T cells)	DK25	DAKO Ltd.	1:100
CD25	IL 2-receptor (TAC-antigen), T-cell activation marker	ACT-1	DAKO Ltd.	1:100
$\gamma\delta$ -TCR	Expressed on small population of normal T cells (<5%)		T Cell Science	1:20
CD45RO	Memory or primed T cells, B-cell subsets, monocytes, macro- phages	UCHL1	DAKO Ltd.	1:200
CD45RA	Naive or virgin T cells, B cells, monocytes		Serotec, UK., Oxford	1:50
CD45R	Leukocyte common antigen, lym- phoid cells	2B11+ PD7/26	DAKO Ltd.	1:100
HLA-DR	MHC class II antigens, expressed on macrophages, B cells, den- dritic cells, activated T cells	CR3/43	DAKO Ltd.	1:100
CD68	Macrophages, monocytes	EBM11	DAKO Ltd.	1:100
CD1A	LCs, cortical thymocytes	NA1/34	DAKO Ltd.	1:100
CD19	Pan B-cell marker	SS2/36	DAKO Ltd.	1:100
CD57	Natural killer cells	HNK-1	BECTON DICKINSON, UK., OXFORD	1:20
MLA	Mucoas Lymphocyte Antigen	BER-ACT8	DAKO Ltd., UK., High Wycombe, Bucks.	1:100

Antibodies were used at concentrations as indicated, diluted in TBS (see Methods). TCR, T-cell receptor.

Milton Keynes, U.K.), mounted on slides coated with 3-aminopropyltriethoxysilane (Sigma Chemical Co., Poole, Dorset, U.K.),²² and air-dried. Endogenous peroxidase activity was blocked with a solution of 0.3% hydrogen peroxide in 1% sodium azide. The slides were washed twice for 5 minutes in 0.05 mol/L Tris-buffered saline (TBS, pH 7.6) and incubated in culture medium (RPMI; Gibco BRL, Paisley, U.K.) containing 20% fetal calf serum and 1% bovine serum albumin for 30 minutes to further prevent nonspecific staining. The slides were drained, and the primary mouse monoclonal antibody (mAb) was applied diluted in TBS as recommended (Table I). One hundred milliliters of diluted mAb was applied to each section and incubated in a humid chamber for 16 to 20 hours. The slides were then washed three times, drained, and incubated with a biotinylated F(ab')₂ fragment of a rabbit anti-mouse immunoglobulin (DAKO Ltd., High Wycombe, Buckinghamshire, U.K.) at a dilution of 1:200 in TBS for 2 hours. After three additional washes, the sections were developed in a peroxidase substrate solution containing 3-amino-9-ethylcarbazole, dimethylformamide, and hydrogen peroxide in 0.1 mol/L acetate buffer.²³ The slides were rinsed in tap water, counterstained in Mayer's hematoxylin, and mounted with glycerol mounting medium (DAKO Ltd.). Conjunctival sections without primary

antibody served as negative controls, whereas tissue sections from tonsils were used as positive controls.

Counting

Staining was evaluated by light microscopy at a total magnification of $\times 400$ with a 0.1 mm² eyepiece graticule. Eight or more adjacent fields were counted and expressed as mean counts per millimeter for the epithelium and mean counts per square millimeter for the subepithelium (plus or minus standard errors). Cell counts were carried out on uncoded slides to avoid bias.

Statistical analysis

Differences between allergic and normal conjunctiva were statistically analyzed by using the nonparametric Mann-Whitney U test.

RESULTS

T cells identified by the presence of cell surface CD3 were present at tarsal and bulbar sites in both normal and diseased conjunctiva. At subepithelial sites of normal conjunctiva, there was great variation in the number of T cells present, ranging from 0 to 100 per square millimeter. However, compared with normal conjunctiva, all the diseased

EXPRESSION OF T CELL MARKERS IN ALLERGIC AND NORMAL CONJUNCTIVA

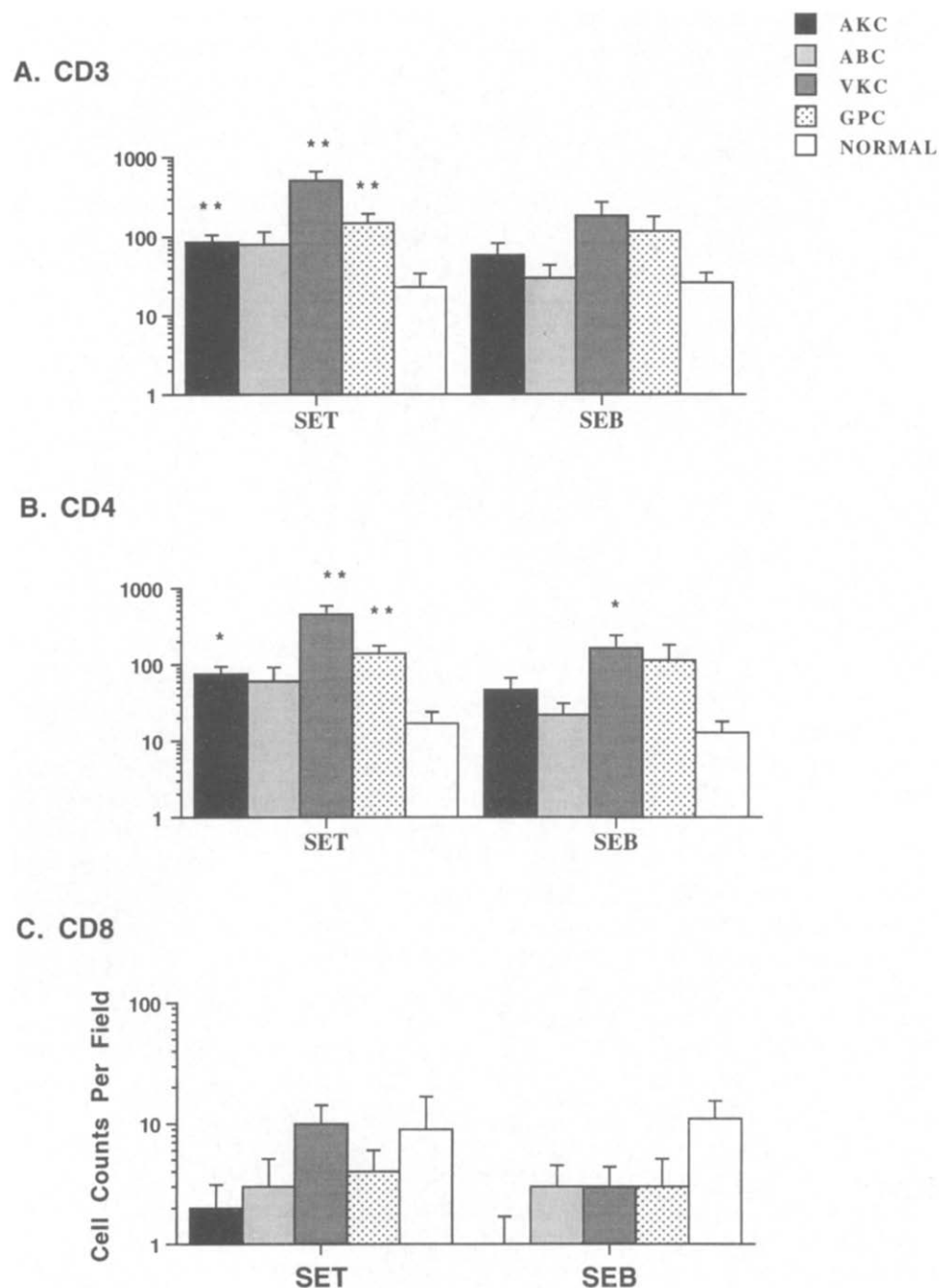


FIG. 1. Expression of T-cell subsets in allergic and normal conjunctiva. Cell counts are expressed as means $\pm$ SEM. Comparisons between chronic allergic and normal conjunctiva were analyzed by using the Mann-Whitney U test. SE, Subepithelium; T, tarsal; B, bulbar. * $p \leq 0.05$, ** $p \leq 0.01$.

groups, with the exception of ABC, had significantly greater numbers of T cells in the subepithelial tarsal conjunctiva ($p < 0.01$) (Figs. 1 and 2). This was particularly evident in biopsy specimens from patients with VKC and GPC, with an average

of 22 times and 6 times as many T cells in tarsal sites, respectively (Fig. 1). The same trend was present in the epithelia, with increased numbers of T cells present in tarsal and bulbar epithelia of specimens from patients with AKC, VKC, and

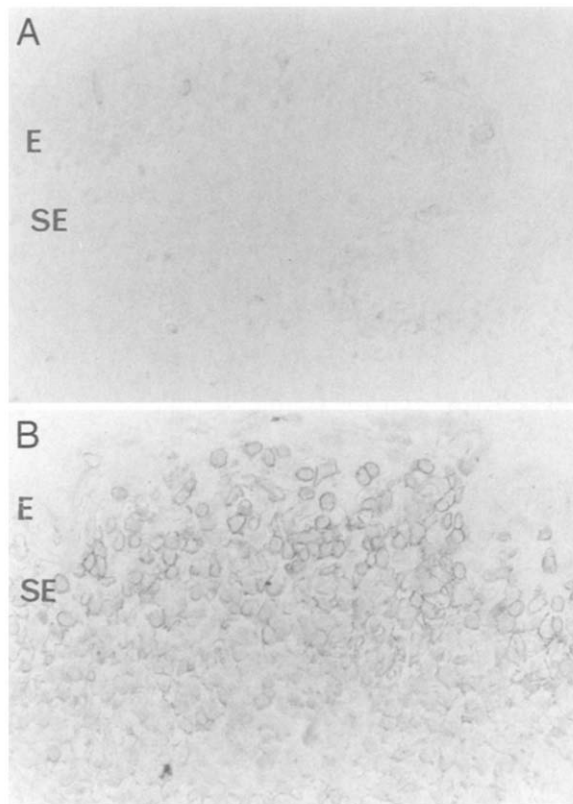


FIG. 2. Tarsal tissue section from normal conjunctiva (**A**) and conjunctiva of patient with VKC (**B**) stained with anti-CD3. E, Epithelium; SE, subepithelium.

GPC compared with normal specimens. CD8-expressing T cells exceeded CD4⁺ cells two- and fourfold in the epithelia of normal tarsal and bulbar conjunctiva, respectively (data not shown). In contrast, the CD4/CD8 ratios in the epithelia of tarsal AKC, VKC, and GPC was markedly altered in favor of CD4⁺ T cells with ratios of 1.5 to 1 in AKC and 1 to 1 in VKC and GPC (data not shown). At subepithelial sites of normal conjunctiva, there were 5 to 10 cells per square millimeter, comprising a mixture of CD4⁺ and CD8⁺ T cells. As in the epithelium, the majority of the infiltrating T cells in subepithelial sites of allergic conjunctiva were of the CD4⁺ phenotype (Fig. 1).

Conjunctival T cells were characterized on the basis of CD45 isoform expression. The high molecular weight isoform CD45RA and the low molecular weight isoform CD45RO, which are believed to represent naive and memory T cells, respectively, were expressed in normal conjunctiva in equal proportions (Fig. 3). In contrast, more than 90% of the infiltrating T cells in all the allergic conjunctival specimens, both in the epithe-

lium (data not shown) and the subepithelium, were of the CD45RO phenotype (Fig. 1, A, and Fig. 3, B and C). Interestingly, 20% to 50% of CD3⁺ T cells present in the epithelium and subepithelium of VKC and GPC co-expressed both isoforms of CD45, based on cell counts (Fig. 3) and morphologic observations in sequential sections (Fig. 4). In contrast, CD45RO⁺RA⁺ cells were rare, if present at all, in AKC and ABC (Fig. 3). Although CD45RO and CD45RA expression was limited to lymphocytes, the leukocyte common antigen, CD45, was prevalent on a wide variety of leukocytes including lymphocytes, macrophages, polymorphonuclear cells, mast cells, and fibroblasts. Leukocyte common antigen expression was more than twice as high as CD3 or CD45RO expression (Fig. 1, A, and Fig. 3, A and B).

CD25 is a component of the IL-2R (Tac or α -chain), and mAbs to CD25 are commonly used as T-cell activation markers. Few cells expressed CD25 in normal and diseased conjunctiva (data not shown). The IL-2R was significantly upregulated in tarsal biopsy specimens from patients with VKC and GPC, although this difference was proportional to the general increase in CD4⁺ T cells in the diseased groups. The percentage of CD25 expression on infiltrating T cells, however, was not significantly different in allergic (23% VKC, 39% GPC) and normal tarsal conjunctiva (35%).

Nonetheless, sequential sections stained for CD3 and HLA-DR revealed strong HLA-DR expression on T cells infiltrating allergic but not normal conjunctiva. In allergic specimens HLA-DR antigens were significantly upregulated in tarsal conjunctiva of patients with AKC, VKC, and GPC (Fig. 5, A). In addition to its expression on T lymphocytes in the allergic groups, HLA-DR was also observed on macrophages (CD68⁺), dendritic cells (CD1A⁺), fibroblasts, and granulocytes (morphologic observation), as well as on endothelial and epithelial cells; whereas in normal conjunctiva epithelial cells rarely expressed HLA-DR (Fig. 6).

Positive staining was also detected for the mucosal lymphocyte antigen (MLA) in the normal conjunctiva (tarsal subepithelium, 2 ± 1.1 ; bulbar subepithelium, 4 ± 2.3) and was significantly increased in the subepithelia of tarsal VKC and GPC (16 ± 5.4 and 34 ± 12.6 , respectively). Sequential sections revealed that these MLA⁺ cells either co-expressed CD4 or were CD4 and CD8 double-negative but never expressed CD8.

CD68-expressing macrophages were seen frequently in normal conjunctiva, with a significant

EXPRESSION OF CD45 ISOFORMS IN ALLERGIC AND NORMAL CONJUNCTIVA

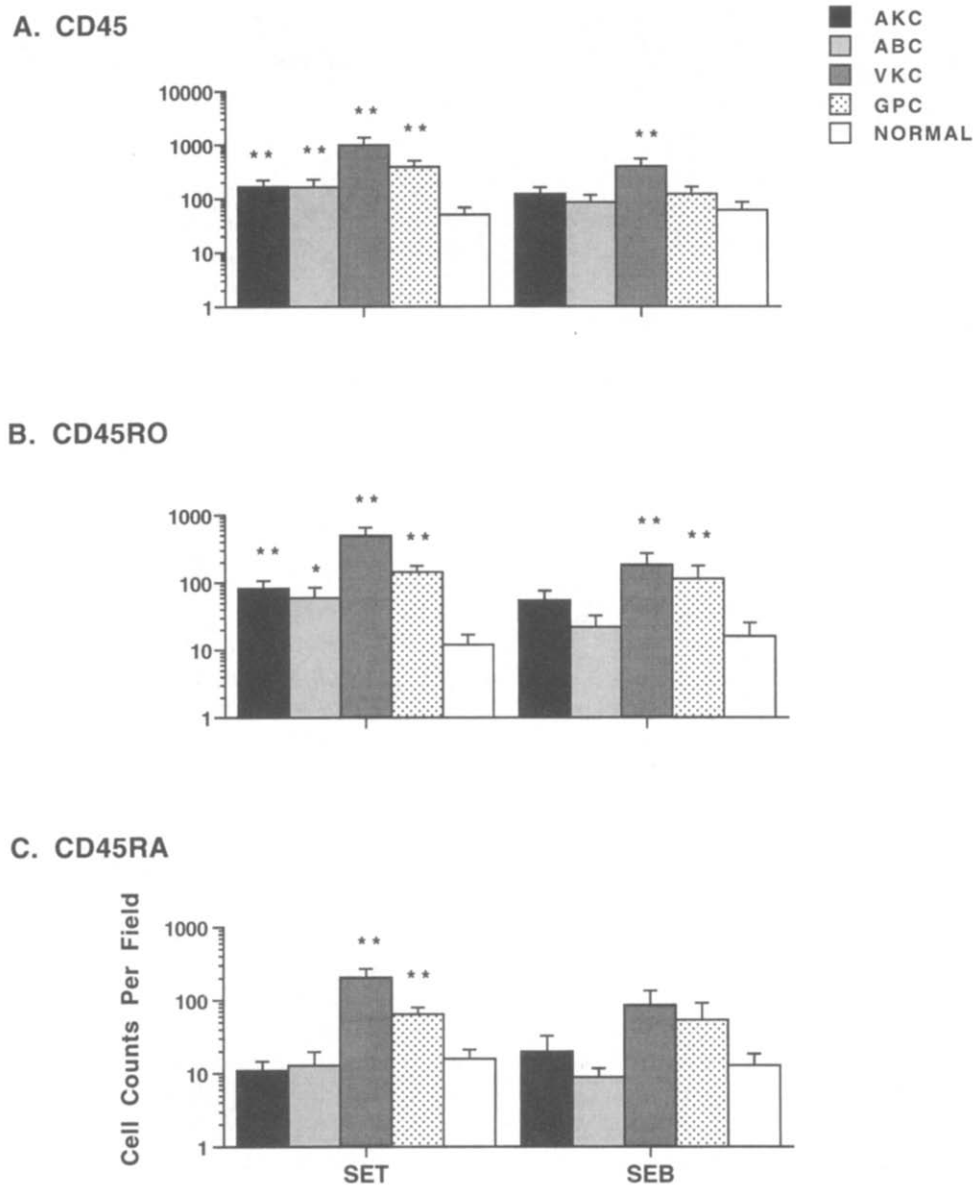


FIG. 3. Expression of different CD45 isoforms in allergic and normal conjunctiva. Cell counts are expressed as means $\pm$ SEM. Comparisons between chronic allergic and normal conjunctiva were analyzed by using the Mann-Whitney U test. *SE*, Subepithelium; *T*, tarsal; *B*, bulbar. * $p \leq 0.05$, ** $p \leq 0.01$.

upregulation in tarsal AKC, VKC, and GPC (Fig. 5, *B*). The numbers of dendritic cells stained with the mAb CD1a, which is prevalent on Langerhans cells (LCs) in particular, were also significantly increased in VKC and GPC (Fig. 5, *C*).

Little difference was observed between allergic

and normal conjunctiva in the expression of the B-cell marker CD19. Similarly, natural killer cells (expressing CD57) and T cells expressing the alternative T-cell receptor $\gamma\delta$ were not found to be different in allergic and normal conjunctiva and were observed in very low numbers in a few tissues only (data not shown).

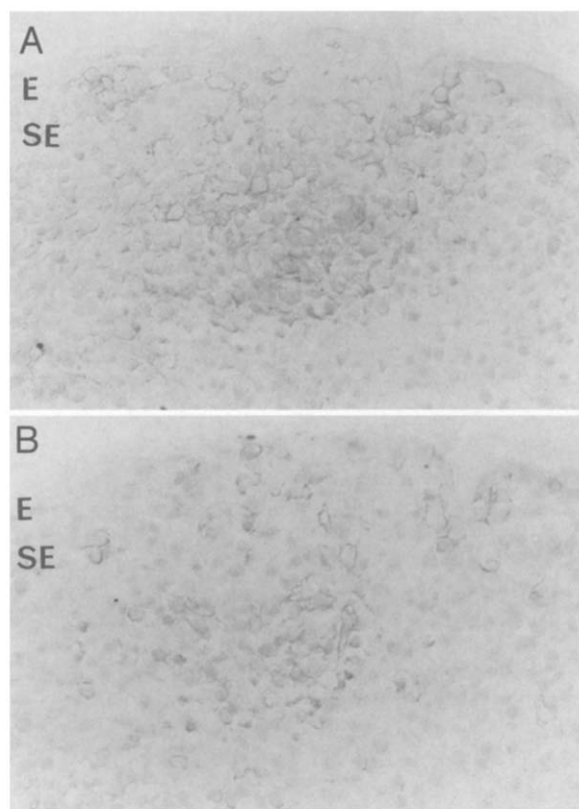


FIG. 4. Serial tissue sections from tarsal conjunctiva of patient with VKC showing: **A**, CD45RO⁺ cells in epithelium and subepithelium; and **B**, CD45RA⁺ cells in the same microscopic field as in **A**. *E*, Epithelium; *SE*, subepithelium.

DISCUSSION

In this study we investigated the expression of T-cell surface antigens to study similarities and differences in T-cell populations infiltrating the conjunctiva of clinically distinct chronic allergic eye conditions. We also investigated the activation status of T cells and the prevalence of different types of antigen presenting cells (APCs).

The cellular infiltrate in chronic allergic conjunctivitis revealed significantly greater numbers of activated (HLA-DR⁺), CD4⁺ memory (CD45RO⁺) T cells in tarsal conjunctiva of patients with VKC, GPC, and AKC than in normal conjunctiva. T cells infiltrating the conjunctiva of patients with ABC were not significantly increased. HLA-DR was also expressed on LCs and macrophages, as well as on other mononuclear cells and endothelial and epithelial cells. The function of HLA-DR-expressing epithelial and endothelial cells is still unknown but implies their possible involvement in antigen presentation to CD4⁺ T cells. Conventional APCs, in particular LCs, were only increased in VKC and GPC, whereas

increased numbers of macrophages were found in all allergic groups except for ABC.

Increased numbers of inflammatory cells were observed in all conditions studied, and it seems likely that a number of these cells were recruited to the site of inflammation nonspecifically because of increased expression of adhesion molecules and the prevalence of cytokines produced by antigen-specific T cells.

However, although a general inflammatory response was characteristic of all four patient groups, VKC and GPC form a group defined by both clinical and pathologic criteria, as do AKC and ABC.

The prevalence of activated CD4⁺ memory T cells characterizes all four chronic allergic eye conditions and suggests that T cells play an important role in the immunopathology observed. It clearly indicates that more than just a mast cell-mediated type I hypersensitivity is involved in all four allergic eye diseases. CD4⁺, CD45RO⁺ T cells have also been reported by Frew and Kay²⁴ and Waldorf et al.²⁵ in the late-phase skin reactions in atopic subjects and in cutaneous delayed type hypersensitivity, respectively.

An additional similarity in all four disorders is the low prevalence of B lymphocytes. Our findings are in agreement with those of El Asrar et al., who also reported a lack of B cells and IgE-producing plasma cells in conjunctival biopsy specimens of patients with AKC.²⁶ This observation would suggest that an immediate hypersensitivity reaction was followed by an infiltration of T_{H1}-like T cells, mediating a cell-mediated immune response. However, the possible involvement of T_{H2} cannot be ruled out, because priming of IgE-producing B cells by IL-4-secreting T lymphocytes may occur in local lymph nodes rather than in the affected tissue itself. High IgE levels in tears and on conjunctival mast cells of patients with AKC and VKC are well documented.^{4, 6, 10, 26, 27}

Differences between AKC and ABC versus VKC and GPC were observed first with regard to the T-cell populations themselves. We found that although 90% of T cells present in all four conditions expressed CD45RO, up to 50% of CD3⁺ T cells infiltrating VKC and GPC co-expressed CD45RO and CD45RA. Such cells were rare or absent from AKC and ABC.

CD45RO/RA double-positive T cells in VKC and GPC could mean one of two things. First of all, because CD4⁺ T cells switch from CD45RA⁺ to CD45RO⁺ during the transition from naive to memory cells, CD45RO/RA double-positive cells

EXPRESSION OF MARKERS ASSOCIATED WITH ANTIGEN PRESENTATION

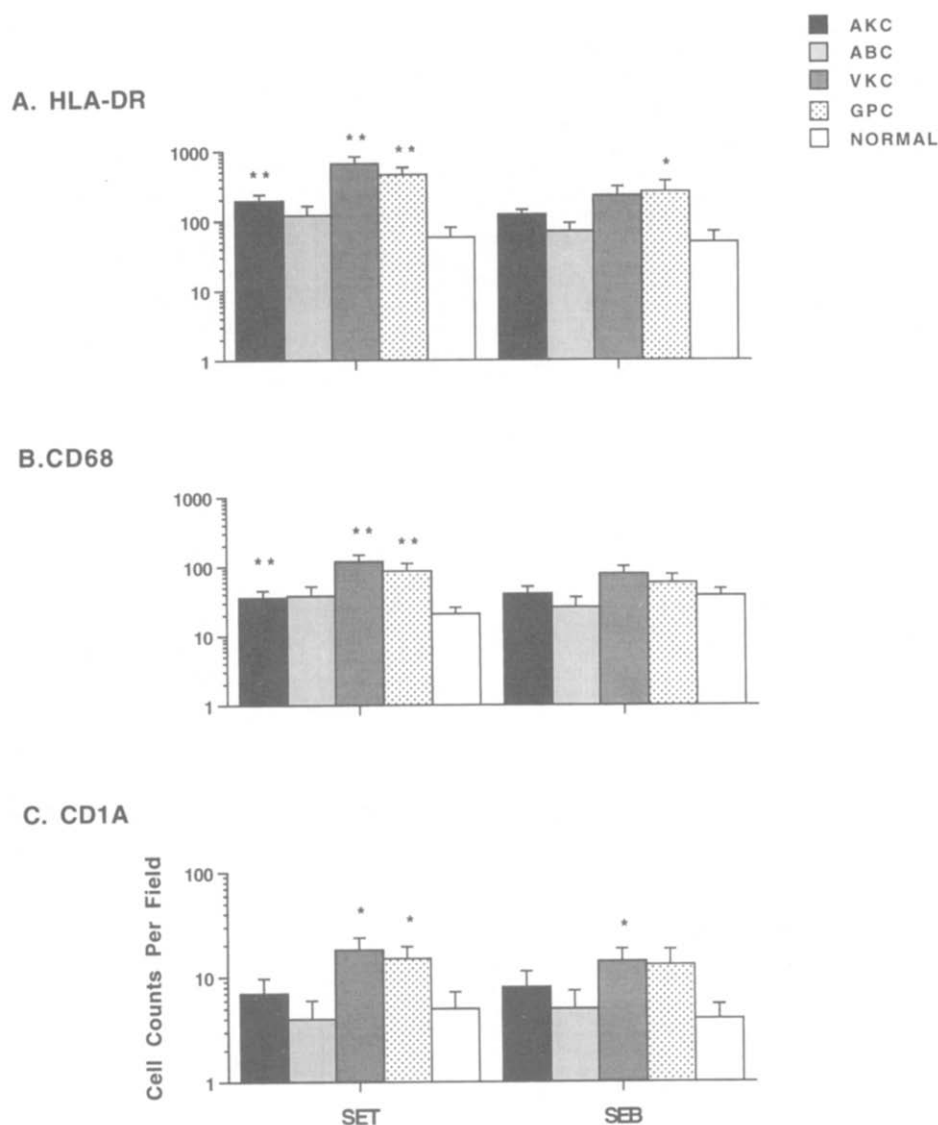


FIG. 5. Expression of HLA-DR, CD68, and CD1A in allergic and normal conjunctiva. Cell counts are expressed as means $\pm$ SEM. Comparisons between chronic allergic and normal conjunctiva were analyzed by using the Mann-Whitney U test. *SE*, Subepithelium; *T*, tarsal; *B*, bulbar. * $p \leq 0.05$, ** $p \leq 0.01$.

in VKC and GPC may represent T cells making such a switch and suggests local T-cell priming. Local T-cell priming was further suggested by an increase in LCs in the epithelia and subepithelia of patients with VKC and GPC. In contrast, patients with AKC showed no increases in the numbers of LCs, and local antigen presentation would have to rely on other HLA-DR⁺ mononuclear cells.

Alternatively, it is conceivable that memory T cells infiltrating AKC and ABC, which are both associated with systemic atopy, may be recruited

from the circulating T-cell pool, which is already engaged in an allergic response, thereby circumventing the need for T-cell priming in the conjunctiva. This would explain the lack of CD45RO/RA double-positive T cells in these conditions.

The second possible explanation for CD45RO/RA double-positive T cells in VKC and GPC stems from the murine system, in which the high and low molecular weight isoforms of CD45 have recently been correlated to functionally distinct T-cell subsets. Luqman et al.²⁸ reported a

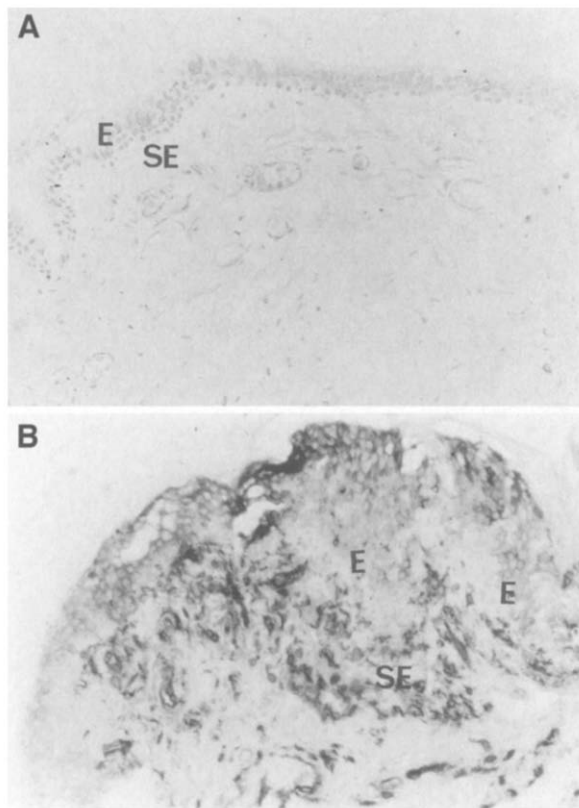


FIG. 6. Tarsal tissue section from normal conjunctiva (**A**) and conjunctiva of patient with GPC (**B**) stained with anti-HLA-DR. E, Epithelium; SE, subepithelium.

correlation between murine CD45RO⁺/RA⁺ T cells and the functional T-cell subset T_{H2}, whereas CD45RO⁺/RA⁻ cells correlated with T_{H1} cells. A phenotypic correlation with functional T-cell subsets defined by their lymphokine production is less well defined in the human system. The majority of human T-cell clones produce a mixed lymphokine profile similar to the mouse T_{H0} clones.²⁹ However, T_{H1} cells producing IL-2 and interferon- γ and T_{H2}-like T cells producing IL-4 and IL-5 have been shown to be present in human beings as the final stage of T-cell differentiation in association with chronic infectious and immunopathologic conditions, including allergy.^{20, 30, 31} Wierenga et al.¹⁷ and Kapsenberg et al.¹⁸ have shown that allergen-specific T-cell clones from atopic donors produce IL-4 but little or no interferon- γ ; whereas non-allergen, antigen-specific clones from the same patient produced interferon- γ but little IL-4. In agreement with the findings of Wierenga et al.,¹⁷ Romagnani et al.³² have cloned T cells that produced IL-4 but virtually no interferon- γ from the conjunctiva of patients with vernal conjunctivitis.

Therefore it is likely that T cells infiltrating the conjunctiva of VKC and GPC may also produce a distinct lymphokine profile similar to the murine T_{H2} and that such T cells possibly co-express CD45RO and CD45RA.

An additional difference observed between VKC and GPC versus AKC and ABC was an increased presence of MLA⁺ lymphocytes in the former. MLA detects the same molecular targets as HML-1 and B-Ly7 on intraepithelial T cells in the gut and was found on up to 50% of T cells in the lamina propria.^{33, 34} As for the possible function of these mucosal lymphocytes in the conjunctiva of VKC and GPC, one may only guess that because of their CD4⁺ and CD45RO⁺ phenotype they are capable of providing help for immunoglobulin synthesis.

An obvious question arising from these observations is why AKC and VKC develop corneal complications and are potentially blinding, whereas ABC and GPC do not. One possible explanation lies in the numbers of T cells present in the respective diseases. T-cell infiltrates were greater in AKC than in ABC and in VKC than in GPC, respectively. Similarly, Bacon et al.²⁷ showed greater numbers of mast cells, eosinophils, and neutrophils in AKC than in ABC, and Udell³⁵ demonstrated greater levels of eosinophil-associated major basic protein in tears of patients with VKC than in those with GPC. The effects of mediators released by degranulating effector cells on host tissue, such as major basic protein on epithelial cells, have been well described.³⁶ Therefore quantitative differences in T cells and effector cells may explain the structural damage to the cornea involved in AKC and VKC but not ABC and GPC.

In summary, this study revealed qualitative differences between AKC and ABC versus VKC and GPC. The presence of HLA-DR⁺, CD45RO single-positive CD4⁺ T cells infiltrating AKC and ABC in the absence of increased professional APCs (dendritic cells, LCs) was in contrast to observations in VKC and GPC, in which up to 50% of CD45RO⁺ T cells co-expressed CD45RA and in addition were associated with an increase in LCs. Two possible explanations were offered for these differences. First, T cells present in AKC and ABC, which are both associated with systemic atopy, may be recruited from the circulation containing allergen-specific memory T cells, thus circumventing the need for antigen priming in the conjunctiva. In contrast, T cells infiltrating VKC and GPC are primed by antigen presented to them in the con-

junctiva. Second, a nonexclusive alternative is that CD45RO single-positive and CD45RO/RA double-positive T cells may represent functionally distinct subsets of memory T cells that require different types of APCs to present their relevant antigen. Quantitative differences between either of the two allergic eye diseases that share a similar spectrum of symptoms may explain corneal complications observed in AKC and VKC, which bear greater numbers of both T cells and effector cells than ABC and GPC, respectively.

The correlation between T-cell numbers and effector cells in chronic allergic eye conditions suggests the involvement of IL-4 and IL-5, which are both secreted by T_{H2}-like T cells. We are presently investigating T-cell lymphokine production in these allergic eye disorders by in situ hybridization. The further characterization of the T-cell subsets involved in regulating the immunopathology of these chronic allergic eye responses will elucidate whether functionally distinct T-cell subsets are involved in AKC and VKC. This may lead to alternative treatment regimens not involving corticosteroids. Immunotherapy, for example, may allow downregulation of the functionally active T-cell subsets or the suppression of T-cell-derived cytokines.

We thank Dr. Karen Britten from Southampton General Hospital for her technique of immunohistochemistry on GMA-embedded tissue sections and Mr. Roger Buckley and John Dart for allowing us to recruit patients from their clinics at Moorfields Eye Hospital.

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