HISTOLOGY AND IMMUNOPATHOLOGY OF SYSTEMIC LUPUS ERYTHEMATOSUS AFFECTING THE CONJUNCTIVA

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SUMMARY

Systemic lupus erythematosus (SLE) is an autoimmune disease occasionally involving the conjunctiva, sclera or cornea. The immunopathology of the active epibulbar lesions has not been studied in detail. Conjunctival biopsies from 11 SLE patients with active epibulbar lesions and from 12 age-matched individuals undergoing cataract surgery were analysed by light microscopy, immunofluorescence and immunoperoxidase techniques. SLE patients presented with scleritis (3 cases), peripheral ulcerative keratitis (5 cases) or progressive cicatrising conjunctivitis (5 cases). Histologically, SLE specimens showed moderate subepithelial and perivascular mononuclear cell infiltration or granuloma formation in the substantia propria, and squamous metaplasia; thrombosis was not seen. Immunoreactant deposition was present at the epithelial basement membrane in 4 of 5 cases with cicatrising conjunctivitis. Vascular immunodeposits were detected in 4 cases. The epithelium showed increased T helper cells (CD4+), granulocytes and natural killer cells (CD67+), dendritic cells (CD1+), and an increase in HLA-DR expression compared with normal tissue. In the substantia propria, B cells (CD22+), macrophages (CD14+), dendritic cells, activated T cells (CD25+, CD3+), the T helper (CD4+)/T suppressor (CD8+) ratio and HLA-DR expression were all increased. These observations suggest that the rare epibulbar manifestations in SLE result from immune-complex-mediated reactions.

Systemic lupus erythematosus (SLE) is an autoimune disease associated with severe alterations in immune regulation. The aetiology may be multifactorial, involving genetic predisposition and environmental factors.[1] The disease is characterised by a great clinical diversity, including facial rash, discoid lupus, Raynaud's phenomenon, alopecia, photosensitivity, oral and nasopharyngeal ulcers, non-deforming arthritis, nephrotic syndrome, pleuritis, pericarditis, psychosis, convulsions, anaemia, leucopenia or thrombocytopenia.[2,3] The course may be acute but frequently is chronic with remissions.

Ocular involvement in SLE is well documented, but the prevalence of ocular manifestations varies in unselected patients, depending on the exacerbations and remissions of the condition.[4,5] Intraocular manifestations, which include uveitis, cotton wool spots and intraretinal haemorrhages, perivasculitis, vasculitis, papilloedema and optic neuritis, are common.[4,6,7] The retinopathy may parallel the activity of the systemic disease,[8,9] being a marker for poor prognosis for survival.[10] Extraocular findings are keratoconjunctivis sicca (KCS),[11] superficial punctate keratopathy,[12] peripheral corneal infiltrates,[13] interstitial keratitis,[14,15] episcleritis[16,17] or scleritis.[18] Conjunctival involvement has been found occasionally.[4,5,19,20]

Only limited data have been published on the histological or immunofluorescent characteristics of conjunctival specimens in SLE patients;[16,21] there has been no characterisation of the mononuclear cells involved. Therefore, we investigated conjunctival specimens from SLE patients by classical histological, immunofluorescent and immunoperoxidase techniques.

MATERIALS AND METHODS

Patients

Conjunctival biopsies from 11 patients with SLE were taken. Twelve conjunctival specimen from healthy adults, obtained during cataract surgery, served as a control group.

The diagnosis of SLE was based on a combination of clinical and laboratory parameters and met the

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Table I. Primary monoclonal antibodies used and their specificities

Antibody	Dilution	Vendor	Specificity
Anti-CD1a	1/40	Dako, Carpinteria, CA	Langerhans cells
Anti-CD3	1/40	AMAC, Westbrook, ME	T cells, thymocytes
Anti-CD4	1/40	AMAC	T helper/inducer cells
Anti-CD8	UD	Becton Dickinson, Mountain View, CA	T cytotoxic/suppressor cells
Anti-CD14	1/40	AMAC	Monocytes/macrophages
Anti-CD22	UD	Becton Dickinson	B cells
Anti-CD25	UD	Becton Dickinson	Activated T cells
Anti-CD67	1/40	AMAC	Granulocytes, natural killer cells
Anti-HLA-DR	1/100	Becton Dickinson	HLA-DR (class II histocompatibility antigen)

UD, undiluted.

criteria for the diagnosis of the disease accepted by the American Rheumatism Association.[2,3] Personal data, symptoms and signs of ocular and systemic problems, and previous and current treatment modalities were recorded. Anterior segment and external ocular photographs recorded the findings of site and degree of external eye inflammation.

Biopsy

After informed consent had been obtained from the patient, the biopsies were taken by a technique previously described.[22] Briefly, the tissue was harvested from the bulbar conjunctiva adjacent to the limbus. In patients with SLE the area of obvious inflammation was chosen. The average size of the specimen was 7 × 4 mm. Each specimen was divided into two equal pieces.

Histopathology

One piece of the specimen was placed in Karnovky's fixative (1% paraformaldehyde, 1.25% glutaraldehyde, 0.13% sucrose, and 25 mM sodium phosphate in 150 mM sodium cacodylate buffer, pH 7.2) and stored overnight at 4 °C. After dehydration in ascending ethanol concentrations, the tissue was embedded in Historesin (LKB-Produkter AB, Bromma, Sweden), sectioned at 2 μm, and stained by standard procedures with haematoxylin and eosin, periodic acid–Schiff and alkaline Giemsa.

Immunofluorescence

The second piece of tissue was snap-frozen in liquid nitrogen, embedded in OCT compound (Tissue Tek, Miles Laboratories, Naperville, IL) and stored at −70 °C until sectioning. Four micrometre cryostat sections were cut onto gelatin-coated 12-well slides for immunofluorescence and immunoperoxidase studies.

Direct immunofluorescent staining was performed using fluorescein (FITC)- or rhodamine (TRITC)-labelled goat IgG directed against each human immunoglobulin (IgA, IgD, IgE, IgG, IgM), complement components C3 and C4, fibrin and albumin (Organon Teknika-Cappel, Durham, NC), as previously described.[22,23] Briefly, antisera were diluted in 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS), pH 7.2 (except for goat anti-human albumin, which was diluted in PBS alone). After air-drying the slides, one drop of conjugated anti-serum was applied and incubated for 30 minutes at room temperature in a moisture chamber. The slides were washed with PBS and coverslipped. Slides were read by a fluorescence microscope (Zeiss Photomic III, Oberkochen, Germany).

Immunoperoxidase

The cryosections were air-dried, fixed in 100% acetone, and incubated with 1% BSA in PBS, pH 7.2, for 30 minutes. Tissue sections were then incubated with the primary antibodies for 45 minutes at room temperature followed by a 20 minute block with 3% H_2O_2 for endogenous peroxidase. Antibodies used in these studies are listed in Table I. Following a series of PBS washes, the tissue was incubated for 30 minutes with a 1:500 dilution of Biotin-SP-AffiniPure goat anti-mouse IgG (H+L) (Jackson ImmunoResearch Laboratories, West Grove, PA). After washing in PBS, the sections were incubated for 20 minutes with a 1:1000 dilution of peroxidase-conjugated streptavidin (Jackson ImmunoResearch Laboratories), and reacted with peroxidase substrate containing 3-amino-9-ethylcarbazole and H_2O_2 in 0.1 M acetate buffer. Specimens were then post-fixed in formalin, counterstained with Gill's No. 3 haematoxylin, and coverslipped. Positive brown reactions on cell surfaces in the substantia propria were counted in two serial sections in masked fashion in three representative high-power fields (×450) with a 10 × 10 mm square grid; a 10 × 2 mm grid was used for counting epithelial cells.

The mean number of stained cells of each cell subset and the standard error were calculated. The significance of differences of means between normals and SLE patients was calculated by a two-tailed Student's *t*-test.

RESULTS

Patients

Table II summarises the clinical characteristics and the treatment modalities of our patients at the time of biopsy. The mean age was 54.6 years (range 17–77 years). Nine patients were female, 2 were male. Five

Table II. Systemic lupus erythematosus: ocular manifestations and treatment at the time of biopsy

Case no.	Sex/age (years)	Manifestation	Treatment
1	F/75	Cicatrising conjunctivitis, episcleritis, SPK, IK	NSAID
2	F/52	Episcleritis, scleritis, PUK	NSAID, DXC, TC
3	F/77	Sicca, PUK, optic neuritis	None
4	M/65	Scleritis	NSAID
5	F/39	Cicatrising conjunctivitis	TC
6	M/62	PUK	NSAID, DXC
7	F/57	Cicatrising conjunctivitis, sicca	NSAID
8	F/50	Cicatrising conjunctivitis, sicca	None
9	F/17	Cicatrising conjunctivitis, IK	TC
10	F/59	Scleritis, PUK	NSAID, hydroxychloroquine
11	F/48	PUK, sicca	NSAID, prednisone

SPK, superficial punctate keratopathy; IK, interstitial keratitis; PUK, peripheral ulcerative keratitis; NSAID, non-steroidal anti-inflammatory drugs; DXC, doxycycline; TC, topical corticosteroids.

patients had cicatrising conjunctivitis, 2 had episcleritis, 3 presented with diffuse scleritis, 4 with sicca syndrome, 5 had peripheral ulcerative keratitis, 1 patient had superficial punctate keratopathy without sicca syndrome, and interstitial keratitis was present in 2 patients. One patient showed optic neuritis. At the time of biopsy, 7 patients were on oral non-steroidal anti-inflammatory drugs, 1 was on chloroquine, 1 patient received prednisone therapy in non-immunosuppressive dosages, and 3 patients were on topical corticosteroids. Two patients with concomitant acne rosacea were on a doxycycline regimen.

Histopathology of SLE

The histological features of our SLE patients are summarised in Table III.

Epithelium. Abnormalities of the epithelium, found in all but 3 patients, consisted of epithelial attenuation (4 patients) and squamous metaplasia (6 patients). The number of globlet cells was diminished in 6 patients and increased in 2. Increased numbers of goblet cells (cases 2 and 4) correlated with high inflammatory activity of an underlying scleritis. Diminished numbers of goblet cells, as well as squamous metaplasia, correlated clinically with progressive cicatrising conjunctivitis (cases 1, 5 and 9), sicca syndrome (case 11), both (case 8), or peripheral ulcerative keratitis (case 6). Epithelial invasion by neutrophils appeared with peripheral ulcerative keratitis in 1 case, and leucocytic epithelial invasion correlated with interstitial keratitis in another.

Substantia propria. Subepithelial mononuclear infiltrates were found in 9 patients. The infiltration was severe in 4 cases. Granuloma formation was apparent in 2 cases: 1 with peripheral ulcerative keratitis and 1 with interstitial keratitis. In 1 case epitheloid cells and giant cells were present. Increased numbers of mast cells were noted in the subepithelial (7 patients) or perivascular (5 patients) area. An abundance of perivascular mast cells correlated with perivasculitis, and the number of subepithelial mast cells was related to the severity of the mononuclear infiltrate. Plasma cells were abundant in 7 patients (cases 1–4, 6, 9, 10).

Vessels. The vessels were unremarkable in 4 patients, and were slightly dilated in 3 patients. Perivasculitis was seen in 4 patients. Active vasculitis with vessel wall necrosis and acute inflammatory infiltration was not seen. Thrombosis was not found in any SLE specimen.

Immunofluorescence of SLE

The patterns of immunoglobulin or complement deposition found in our SLE patients are shown in Table IV. In the control specimens, the superficial epithelial cells were stained with IgA and IgG, but no further fluorescent staining was detected with the other antibodies used.

Epithelium. Immunoreactant deposition was found in the epithelium in all but 4 patients, with speckled cellular staining in 3 cases. This was seen with IgG and C4 in 2 patients and with IgE in another. At the basement membrane zone (BMZ) 2 specimens were positive for IgG, 2 for C4, 3 for IgA and 1 for each

Table III. Systemic lupus erythematosus; histopathology in conjunctiva

Case no.	Goblet cells (PAS)	Mast cells[a] (Giemsa)	Epithelium (HE)	Substantia propria[a] (HE)	Vessels (HE)
1	None	Normal	Squamous metaplasia	1+ inflammatory infiltrate	Normal
2	3+	1+ perivascular	Attenuated	1+ inflammatory infiltrate	Dilated
3	Normal	2+	Attenuated, 4+ neutrophils	3+ inflammatory infiltrate	Perivasculitis
4	3+	Normal	Normal	2+ inflammatory infiltrate	Perivasculitis
5	None	Normal	Squamous metaplasia	1+ inflammatory infiltrate	Normal
6	Few	2+ perivascular	Squamous metaplasia, attenuated	Granuloma	Normal
7	Normal	Normal	Normal	Normal	Dilated
8	None	1+	Squamous metaplasia	Normal	Dilated
9	None	3+ perivascular	Squamous metaplasia, 2+ lymphocytes	Granuloma	Perivasculitis
10	Normal	2+ perivascular	Normal	1+ inflammatory infiltrate	Perivasculitis
11	Few	2+ perivascular	squamous metaplasia, attenuated	1+ inflammatory infiltrate	Normal

PAS, periodic acid–Schiff staining; HE, haematoxylin–eosin staining. HE (cells/mm^2): 1+, 10–100; 2+, 101–200; 3+, >200. Mast cells (cells/mm^2): 1+, 1–5; 2+, 6–10; 3+, >10. [a]Inflammation located subepithelially.

Table IV. Systemic lupus erythematosus: immunofluorescence in conjunctiva

Case no.	Epithelial cells	Epithelial BMZ			Substantia propria	Vessels
		Speckled	Linear	Granular		
1	IgG, C4	IgG, IgM, C4	–	IgA, C4	–	–
2	IgA, IgD		IgG	–	IgA, IgG, IgM C3	–
3	IgA, IgG		–	–	IgA, IgD, IgG, C3	C4
4	ND		ND	ND	ND	ND
5	–		IgM, C4	–	IgA, IgG	–
6	IgA, IgG		–	–	IgD, IgE, IgG	–
7	IgA, IgD, IgG, IgM, C4	IgE	IgA	IgE	IgA, IgG	IgD
8	IgA, IgG	IgG, C4	IgA, IgG	–	C4	IgM, C3, C4
9	–		–	–	IgA, IgG, C4	C4
10	IgG, C4		–	–	IgG	–
11	–		–	–	IgD, IgG	–

BMZ, basement membrane zone; ND, not determined.

IgM and IgE. Immunodeposition at the BMZ was linear in 4 specimens (Fig. 1) and granular in 2 others. In cases 1, 5, 7 and 8, the deposition of immunoreactants at the BMZ was related to the clinical appearance of progressive cicatrising conjunctivitis.

Substantia propria, vessels. The substantia propria was positive by immunofluorescence microscopy for various immunoglobulins and complement components in all but one specimen. Vessel walls were positive for C4 in 3 cases and for IgD, IgM or C3 in 1 specimen each. Two specimens with perivasculitis (cases 3 and 9) showed vascular deposition of immunoreactants.

Immunoperoxidase of SLE and normals

The mean sizes of mononuclear cell populations identified by monoclonal antibodies in the conjunctival epithelium and substantia propria are shown in Tables V and VI, respectively.

Epithelium. In the epithelium of SLE specimens, the number of CD4+ T cells (T helper/inducer) was significantly increased compared with the control specimens ($p<0.03$), and the CD4/CD8 ratio in SLE patients was higher (3.1:1) than in normals (1:1.85). While the epithelium was negative for CD67-positive cells (granulocytes/natural killer cells) and CD22+ cells (B lymphocytes) in the control tissues, such cells were detected in SLE patients. HLA-DR staining was intensely positive in the epithelium of SLE specimens, not permitting a precise quantitation of positively stained cells. In contrast, only a few HLA-DR expressing cells were seen in the epithelium of the control tissues, and no diffuse epithelial staining was observed (Table V).

Substantia propria. Two control specimens were negative for CD3+, CD4+ and CD8+ cells in the substantia propria, and 10 specimens had scattered positive cells for CD3 (6.45 ± 1.28), CD4 (7.33 ± 0.73) or CD8 (6.04 ± 1.23). Scattered CD25+ cells were detected in the substantia propria of 4 specimens in the control group (2.0 ± 0.44), while the other control tissues were negative for these cell. The CD4/CD8 ratio was 1.2:1 in the substantia propria in normals. In contrast, large numbers of T cells were seen in the substantia propria from patients with SLE (Table VI). The most frequent positive cell type expressed CD3 (74.88 ± 27.45), and many cells expressed CD4 (71.59 ± 17.41; Fig. 2a) or CD8 (21.37 ± 7.26; Fig. 2b). Many more activated T cells (CD25) were present in SLE conjunctiva (13.82 ± 3.55) than in normal conjunctiva. The CD4/CD8 ratio was markedly increased compared with normal individuals (3.35:1).

While only a few CD22+ cells (B cells) were found in the substantia propria in normal tissue (1.73 ± 0.56), significantly more such cells were found in SLE patients (19.8 ± 4.59; Fig. 2c).

In the substantia propria from SLE patients, macrophages (CD14) were significantly increased

Table V. Systemic lupus erythematosus: mononuclear cells in conjunctival epithelium

Cell type	Control[a]	SLE[a]	Significance
CD1a	1.76 ± 0.45	3.77 ± 1.10	NS
CD3	4.71 ± 0.89	8.18 ± 2.32	NS
CD4	2.53 ± 0.66	8.41 ± 1.97	$p<0.03$
CD8	4.69 ± 0.77	2.70 ± 0.74	NS
CD14	0.44 ± 0.29	3.29 ± 0.95	NS
CD22	0 ± 0	6.87 ± 2.26	NS
CD25	0 ± 0	2.14 ± 0.66	NS
CD67	0 ± 0	3.71 ± 0.65	$p<0.05$
HLA-DR	7.27 ± 1.45	Diffuse staining	

[a]Mean cell counts/mm^2 per high-power (×450) ± standard error of the mean.

Table VI. Systemic lupus erythematosus: mononuclear cells in conjunctival substantia propria

Cell type	Control[a]	SLE[a]	Significance
CD1a	1.9 ± 0.75	16.19 ± 3.17	$p<0.01$
CD3	6.45 ± 1.28	74.88 ± 27.45	$p<0.05$
CD4	7.33 ± 0.73	71.59 ± 17.41	$p<0.001$
CD8	6.04 ± 1.23	21.37 ± 7.26	$p<0.05$
CD14	4.56 ± 0.86	17.19 ± 3.56	$p<0.005$
CD22	1.73 ± 0.56	19.8 ± 4.59	$p<0.005$
CD25	2.0 ± 0.44	13.82 ± 3.55	$p<0.02$
CD67	8.17 ± 1.63	10.39 ± 1.93	NS
HLA-DR	9.76 ± 1.01	Diffuse staining	

[a]Mean cell counts/mm^2 per high-power (×450) ± standard error of the mean.

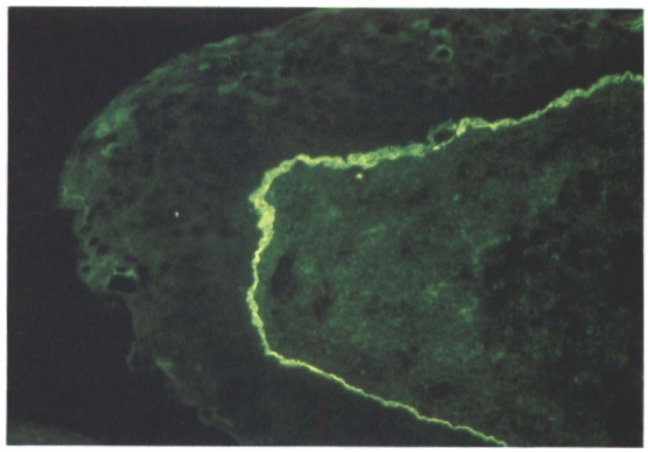
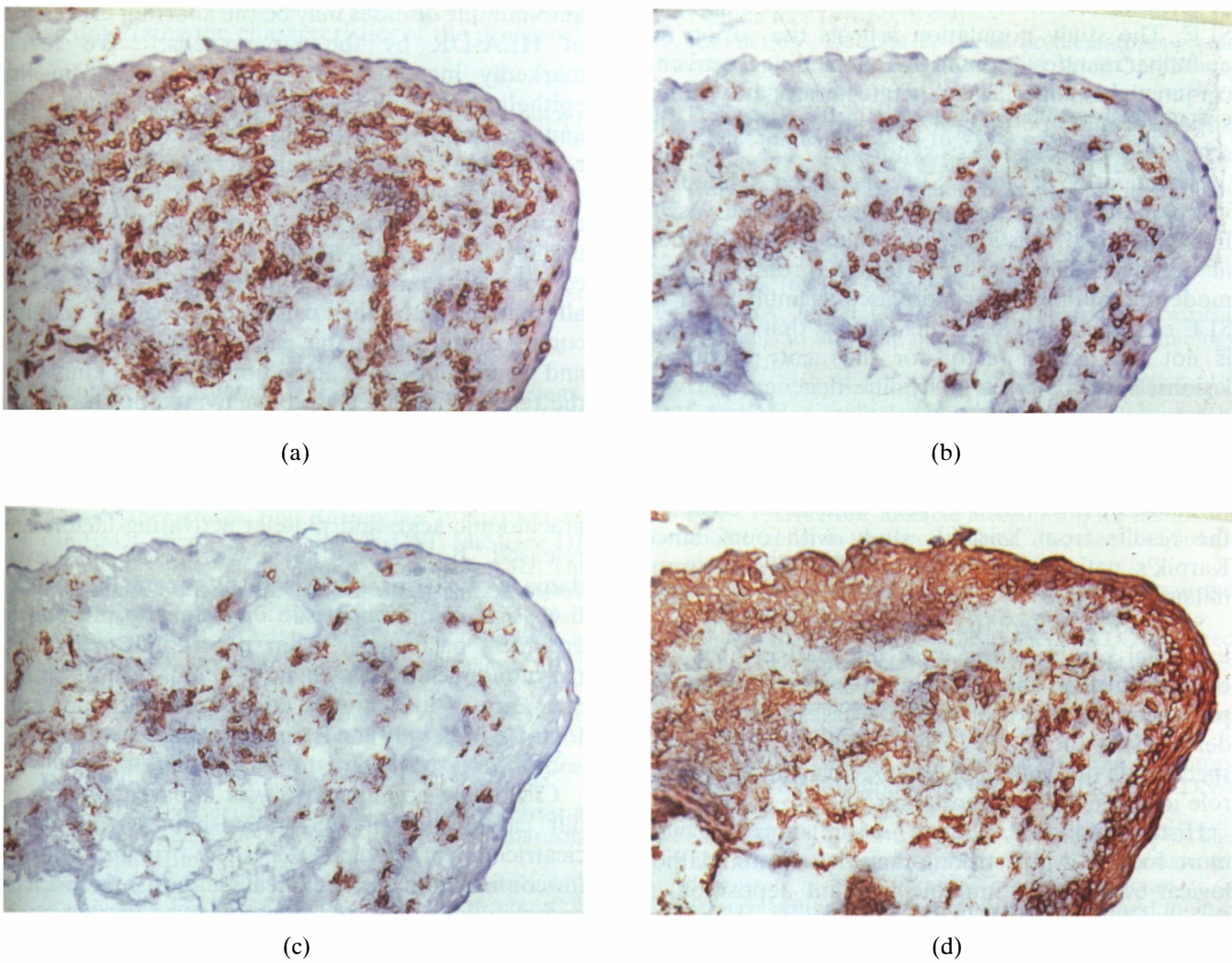


Fig. 1. *Conjunctiva from a patient with systemic lupus erythematosus. Direct immunofluorescence; tissue incubated with FITC-labelled goat-anti-human Iga. Note the positive staining of the basement membrane zone (original magnification ×100).*



(a) (b)

(c) (d)

Fig. 2. *Conjunctiva from a patient with systemic lupus erythematosus. Immunoperoxidase technique (original magnification ×450). Sections were incubated with (a) anti-CD4 (T helper/inducer cells) and (b) anti-CD8 (T cytotoxic/suppressor cells); note the increased subepithelial numbers of CD4 and CD8 cells with a helper/suppressor ratio greater than 1. (c) Section incubated with anti-CD22 (B cells); there are a significant number of B cells in the substantia propria. (d) Section incubated with anti-HLA-DR (class II histocompatibility antigen); note the diffuse and extensive staining in the epithelium and substantia propria, and the positive vessel walls.*

(17.19 ± 3.54) compared with the controls (4.56 ± 0.86). The expression of CD67, expressed on natural killer cells and granulocytes, did not differ between the normals and SLE specimens.

The number of Langerhans cells (CD1) found in the substantia propria was significantly less in control conjunctiva (1.9 ± 0.75) than in SLE specimens (16.19 ± 3.17). Only a few HLA-DR positive cells were seen in the control tissue, but HLA-DR expression was heavily upregulated in SLE patients (Fig. 2d). The HLA-DR staining pattern appeared diffuse in 6 cases, and the positively stained cells were increased overall as compared with normal individuals.

DISCUSSION

This is the first detailed immunopathological characterisation of actively inflamed conjunctival tissue in SLE. The study population reflects the variety of epibulbar manifestations in SLE, including cicatrising conjunctivitis, superficial punctate keratopathy, interstitial keratitis, peripheral ulcerative keratitis, episcleritis, scleritis and sicca syndrome.

Retinopathy in acute SLE has been related to vascular occlusions in the absence of active vasculitis.[24] In a previous study on ocular tissue obtained at autopsy, Karpik *et al.*[21] found thrombosis and moderate mononuclear perivascular infiltration in SLE conjunctiva. Our results suggest that ischaemia is not the critical factor for the acute epibulbar lesions, as thrombosis or hyaline degeneration was not found in any conjunctival tissue analysed. The histological features were subepithelial and perivascular cellular infiltration, or even frank granuloma formation. However, it is very difficult to compare the results from Karpik's study with ours, since Karpik's patients exhibited no clinically apparent inflammation.

Squamous metaplasia, loss of goblet cells and epithelial keratinisation were detected in 6 of our cases. While squamous metaplasia was related to the presence of sicca syndrome in 2 cases, this was not detected in the others. This supports a previous speculation that inflammation may play an important role in epithelial differentiation.[25]

Histopathological evidence of inflammation was more focal and less striking than the immunopathological evidence of immune reactant deposition, a finding which is supported by others.[21] The presence of immunodeposits in some patients with episcleritis, and their absence in others with recent episcleritis, may relate to response to therapy.[16,26] However, immunodeposits have been found in clinically uninvolved conjunctiva.[16,21]

Central to the development of SLE is the production of autoantibodies reactive with membrane molecules, cytoplasmic proteins and nuclear determinants.[27–30] In addition, defective T cell function and regulation can be found in patients with active disease,[31–37] serving as mechanisms for the perpetuation of disease processes. In our study, T cells were greatly increased in the actively inflamed tissue, reflecting T cell participation at the site of tissue damage. Many T cells were activated (IL-2R+), and the CD4/CD8 ratio was markedly increased.

The major histocompatibility complex (MHC) class II molecules of the cell surfaces are associated especially with B cells, antigen presenting cells and macrophages. Gamma interferon, a lymphokine produced by activated T cells, is one of the most common and potent inducers of HLA-DR expression (MHC class II) on epithelial, endothelial and connective tissue cells, including human fibroblasts. It has been suggested that the initial event in autoimmune diseases may be the aberrant expression of HLA-DR by the target cells.[38] We found markedly increased HLA-DR expression in the epithelium and substantia propria of the conjunctiva, and the numbers of antigen presenting Langerhans cells (CD1a) were markedly increased.

In SLE, immune complexes are formed in the circulation and elsewhere, then deposited in certain organs and tissue locations. Activation of the complement pathway, through both classic and alternative routes, then causes, at the site of immune complex deposition, the chemotaxis of neutrophils and macrophages and activation of various kinins. As the result of IL-1 release by activated macrophages, T cells produce IL-2, which has a direct effect on amplifying the proliferation of other T cells. Finally, proteolytic enzymes, oxygen radicals, products of arachidonic acid, and platelet activating factors are released. It appears that the bulk of the tissue damage occurring in SLE results from these reactions and also from tissue digestion by proteolytic enzymes, particularly from neutrophils. However, the mononuclear phagocytic system is critical for the clearance of the immune complexes. The mast cells found in our study can intimately interact with T cells and fibroblasts, leading to scar formation.

Cicatrising conjunctivitis was apparent in five of our cases, clinically indistinguishable from ocular cicatricial pemphigoid (OCP). The primary criterion for confirmation of the clinical diagnosis of cicatricial pemphigoid is the linear deposition of immunoreactants along the BMZ.[22,23] Immunoreactant deposition at the BMZ from conjunctival lesions in SLE patients was usually granular.[21,22] However, linear depositions of immunoreactants similar to those found in OCP were detected in 5 of 12 SLE patients with clinically uninvolved conjunctiva by Burge *et al.*[16] and in 4 of our patients with actively inflamed conjunctiva.

The B cells involved in OCP basically were 'end-stage' B cells (plasma cells) in our recent study,[23] suggesting extraocular activation and maturation of B cells, with subsequent homing to target mucous membranes. In light of the polyclonal B cell activation recently found in SLE,[30] and the presence of HLA-DR controlled immune responses, the abundance of B cells (CD22+) in the active epibulbar lesions is not surprising. However, it is important to distinguish between those immune abnormalities which are inherent to the disease process and those which occur as a consequence of disease. The latter are found especially or entirely during periods of disease exacerbation. Therefore, studies of severe inflammation have limitations with regard to the determination of the defects which preceded or induced disease activity. It may not be primarily the composition of the cellular infiltration, but rather the differences in the secretory activity of these cells, which determine the characteristics of the diseases.[39]

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Key words: Conjunctiva, Systemic lupus erythematosus, Immunopathology, Cicatrising conjunctivitis.

REFERENCES

1. Steinberg AD, Klinman DM. Pathogenesis of systemic lupus erythematosus. Rheum Dis Clin North Am 1988; 14:25–41.
2. Cohen AS, Reynolds WE, Franklin EC, *et al*. Preliminary criteria for the classification of systemic lupus erythematosus. Bull Rheum Dis 1971;21:643–8.
3. Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, Schaller JG, Talal N, Winchster RJ. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25:1271–7.
4. Gold DH, Morris DA, Henkind P. Ocular findings in systemic lupus erythematosus. Br J Ophthalmol 1972; 56:800–4.
5. Harvey AM, Shulman LE, Tumulty PA, *et al*. Systemic lupus erythematosus: review of the literature and clinical analysis of 138 cases. Medicine 1954;33:291–437.
6. Gold DH, Feiner L, Henkind P. Retinal arterial occlusive disease in systemic lupus erythematosus. Arch Ophthalmol 1977;95:1580–5.
7. Deutsch TA, Corwin HL. Lupus optic neuritis with negative serology. Ann Ophthalmol 1988;20:383–4.
8. Committee on prognosis studies in SLE: prognosis studies in SLE: an activity index. Arthritis Rheum 1986;29(Suppl 4):93.
9. Klinkhoff AV, Beattie CW, Chalmers A. Retinopathy in systemic lupus erythematosus: relationship to disease activity. Arthritis Rheum 1986;29:1152–6.
10. Stafford-Brady FJ, Urowitz MB, Gladman DD, Easterbrook M. Lupus retinopathy: patterns, associations, and prognosis. Arthritis Rheum 1988;31:1105–10.
11. Hochberg MC, Boyd RB, Ahearn JM. Systemic lupus erythematosus: review of clinico-laboratory features and immunogenetic markers in 150 patients with emphasis on demographic subsets. Medicine 1985; 64:285–95.
12. Spaeth GL. Corneal staining in systemic lupus erythematosus. N Engl J Med 1967;276:1168–71.
13. Grayson M. Diseases of the cornea. St Louis: CV Mosby, 1979.
14. Reeves JA. Keratopathy associated with systemic lupus erythematosus. Arch Ophthalmol 1965;74:159–60.
15. Williams B, Hull DS. Lupus erythematosus keratoconjunctivitis. South Med J 1986;79:631–2.
16. Burge SM, Frith PA, Juniper RP, Wojnarowska F. Mucosal involvement in systemic and chronic cutaneous lupus erythematosus. Br J Dermatol 1989;121: 727–41.
17. Frith PA, Burge SM, Millard PR, Wojnarowska F. External ocular findings in lupus erythematosus: a clinical and immunopathological study. Br J Ophthalmol 1990;74:163–7.
18. Watson PG, Hayreh SS. Scleritis and episcleritis. Br J Ophthalmol 1976;60;163–91.
19. Klauder JV, DeLong P. Lupus erythematosus of the conjunctiva, eyelids and lid margins. Arch Ophthalmol 1932;71:856–67.
20. DeLong P, Klauder JV. LE of eyelids and conjunctiva. Arch Ophthalmol 1936;16:321–2.
21. Karpik AG, Schwartz MM, Dickey LE, Streeten BW, Roberts JL. Ocular immune reactants in patients dying with systemic lupus erythematosus. Clin Immunol Immunopathol 1985;35:295–312.
22. Foster CS. Cicatricial pemphigoid. Trans Am Ophthalmol Soc 1986;84:527–663.
23. Rice BA, Foster CS. Immunopathology of cicatricial pemphigoid affecting the conjunctiva. Ophthalmology 1990;97:1476–83.
24. Graham EM, Spalton DJ, Barnard RO, Garner A, Russell R. Cerebral and retinal vascular changes in systemic lupus erythematosus. Ophthalmology 1985;92: 444–8.
25. Tseng SCG, Hirst LW, Maumanee AE, Kenyon KR, Sun TT, Green R. Possible mechanisms for the loss of goblet cells in mucin-deficient disorders. Ophthalmology 1984;91:545–52.
26. Jacobs MI, Schned ES, Bystryn JC. Variability of the lupus band test. Arch Dermatol 1983;119:883–9.
27. Blaese RM, Grayson J, Steinberg AD. Elevated immunoglobulin secreting cells in the blood of patients with active systemic lupus erythematosus: correlation of laboratory and clinical assessment of disease activity. Am J Med 1980;69:345–50.
28. Laskin CA, Haddad G, Soloninka CA. The regulatory role of NZB T lymphocytes in production of anti-DNA antibodies *in vitro*. J Immunol 1986;137:1867–73.
29. Kallenberg CG, Limburg PC, Van Slochteren C. The TH B cell activity in systemic lupus erythematosus: depressed *in vivo* humoral immune response to a primary antigen (haemocyanin) and increased *in vitro* spontaneous immunoglobulin synthesis. Clin Exp Immunol 1983;53:371–83.
30. Klinman DM, Ishigatsubo Y, Steinberg AD. Pathogenesis of generalised autoimmunity: *in vivo* studies of the interaction between murine B cells and their internal milieu. J Immunol 1987;139:2284–9.
31. Miller KB, Schwartz RS. Familial abnormalities of suppressor cell function in systemic lupus erythematosus. N Engl J Med 1979;301:903–9.
32. Raziuddin S, Danial HB, Kelley M. OKT4 T cell

abnormality in patients with systemic lupus erythematosus: HLA-DR antigen expressions. Clin Immunol Immunopathol 1988;48:42–9.
33. Morimoto C, Steinberg AD, Letvin NL. A defect of immunoregulatory T cell subsets in systemic lupus erythematosus patients demonstrated with anti-2H4 antibody. J Clin Invest 1987;79:762–8.
34. Sakane T, Steinberg AD, Green I. Failure of autologous mixed lymphocyte reactions between T and non-T cells in patients with systemic lupus erythematosus. Proc Natl Acad Sci USA 1978;75:3464–8.
35. Linker-Israeli M, Bakke AC, Kitridon RC. Defective production of interleukin 1 and interleukin 2 in patients with systemic lupus erythematosus. J Immunol 1983;130:2651–5.
36. Yu DTY, Winchester RJ, Fu SM. Peripheral blood Ia positive T cells: increases in certain diseases and after immunization. J Exp Med 1980;151:91–6.
37. Steinberg AD, Huston DP, Taurog JD. The cellular and genetic basis of murine lupus. Immunol Rev 1981; 55:121–54.
38. Mason DW, Dallman M, Barclay AN. Graft versus host disease induces expression of Ia antigen in rat epidermal cells and gut epithelium. Nature 1981;293: 150–1.
39. Bernauer W, Wright P, Dart JK, Leonard JN, Lightman S. Cytokines in the conjunctiva of acute and chronic mucous membrane pemphigoid: an immunohistochemical analysis. Graefes Arch Clin Exp Ophthalmol 1993;231:563–70.