Expression of CD40 and CD40 Ligand in the Human Conjunctival Epithelium

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Purpose. CD40 antigen is a membrane receptor that plays a role in the regulation of immune reactions. The expressions of CD40 and CD40 ligand (CD40L) were investigated ex vivo and in vitro in conjunctival epithelial cells, in correlation with HLA DR class II antigen, previously shown to be upregulated in conjunctival inflammatory conditions.

Methods. Impression cytology specimens were collected in 186 patients: 52 normal ones, 65 with keratoconjunctivitis sicca, and 69 with chronic conjunctivitis. Cells were processed for flow cytometry, by using monoclonal antibodies to CD40, CD40L, and HLA DR antigens. Chang conjunctival cells were also used and treated with human recombinant interferon (IFN)-γ or tumor necrosis factor (TNF)-α. CD40, CD40L, and HLA DR expressions were studied by flow cytometry after 24 and 48 hours of treatment.

Results. CD40 was found in both normal and pathologic eyes. Quantitation of levels of fluorescence showed a significantly higher expression in pathologic eyes than in normal ones ($P < 0.0001$). CD40L was variably and inconstantly expressed by conjunctival cells. A strong expression of HLA DR was observed in pathologic eyes, whereas normal eyes showed very low levels ($P < 0.0001$). Significantly positive correlations were found among CD40, CD40L, and HLA DR levels. Chang conjunctival cells expressed CD40 in basal conditions, whereas CD40L and HLA DR were negative. CD40 expression significantly increased after 24 hours of IFNγ treatment and after 48 hours' exposure to TNFα. These cytokines had no effect on CD40L expression. HLA DR was upregulated after 24 hours of treatment with IFNγ but remained negative after exposure to TNFα.

Conclusions. Human conjunctival epithelial cells normally express CD40 antigen, and, more inconsistently, CD40L. Flow cytometry showed higher expression of these molecules in inflammatory eyes than in normal ones in correlation with class II antigen expression, as well as CD40 and HLA DR upregulation after treatment with proinflammatory cytokines in vitro. (*Invest Ophthalmol Vis Sci.* 2000;41:120–126)

CD40 is a cell surface receptor that belongs to the tumor necrosis factor receptor (TNF-R) superfamily.[1] Structurally, the CD40 antigen, a phosphorylated 48-kDa glycoprotein is a typical type I transmembrane protein.[2] It was first identified and functionally characterized on B lymphocytes and carcinoma cells.[3] CD40 is an important mitogenic receptor on B lymphocytes that regulates proliferation and differentiation through binding to its ligand (CD40 ligand [CD40L]).

However, in the recent years CD40 has been found to be expressed on many other cells, including monocytes-macrophages, dendritic cells, hematopoietic progenitors, fibroblasts, and endothelial and epithelial cells.[4–7] CD40 is now thought to play an important role in the regulation of the immune responses, chronic inflammation, and angiogenesis.[8] CD40 upregulation has been observed in inflammatory conditions in various tissues and cell systems, especially in occasional antigen-presenting cells such as skin keratinocytes, retinal pigment epithelial cells, thyroid fibroblasts, or endothelial cells.[9–13] The expression of CD40 by these cells indicates the possibility of stimulatory cross-talk between immunocompetent cells bearing CD40L (T-cells, mastocytes) and the target tissue during the inflammatory process. However, CD40–CD40L interaction alone seems to not to be sufficient to provide a mitogenic signal to the T cells and is instead implicated in amplifying the inflammatory reaction.[9] Consistently, in several in vitro models, CD40 increased after cell exposure to interferon (IFN)-γ, a T helper 1 inflammatory cytokine.[11,13,14] Nevertheless, the stimulatory effect of other inflammation-related mediators such as TNFα or interleukin (IL)-1 has been reported to be less constant.[13,14]

The biologic functions of CD40 expressed on nonhematopoietic cells remains unclear, and little is known about the expression of CD40 in the ocular surface. CD40 is expressed on normal human limbal epithelial cells, stromal corneal cells, and cultured human corneal epithelial cells in proliferative stages, but not on corneal endothelial cells.[15] Therefore, it has been suggested that CD40 expression on corneal epithelial cells could be correlated with high proliferative potential.

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Nevertheless, CD40 expression has never been described on human conjunctival epithelial cells, and the functional consequences of CD40 activation on these cells are unknown.

Conjunctival cells are directly involved in inflammatory processes, together with a potent network of immunocompetent cells. It has therefore been clearly shown that inflammatory disorders cause an overexpression of class II antigens HLA DR in conjunctival epithelial cells[16–19] and costimulate apoptosis-related markers.[20] Thus, we investigated in impression cytology specimens the expression of CD40 and CD40L and correlated them with the expressions of HLA DR class II antigens in normal and pathologic conjunctivae. To improve reliability of conjunctival immunocytology, we used a new objective method of flow cytometry in impression cytology.[20,21] Furthermore, in an in vitro model of Chang conjunctival cells we studied CD40, CD40L, and HLA DR expressions at the basal level and after exposure to two inflammatory cytokines, IFNγ and TNFα.

Materials and Methods

Conjunctival Cell Collection

Impression cytology specimens were collected in 186 patients (238 eyes) aged 30 to 80 years. Fifty-two patients (104 eyes) were considered normal (without any ocular surface disorders) according to clinical history and complete biomicroscopic examination. They had not received any topical treatment for at least 2 months before impression cytology. Sixty-nine patients (69 eyes) had chronic conjunctivitis of various origins (contact lens wearing, chlamydial infection, or long-term use of topical drugs). All these patients had had chronic conjunctivitis with clinical inflammation (hyperemia, conjunctival follicles with occasional mild superficial keratitis) for more than 6 weeks. They had received different topical treatments (antibiotics or antiallergic drugs—i.e., antihistamine, mast cell stabilizers) but no steroidal or nonsteroidal anti-inflammatory agents for at least 2 months. Sixty-five patients (65 eyes) had moderate to severe dry eyes characterized by low Schirmer test results (less than 6 mm at 5 minutes), profoundly lowered break-up time (less than 6 seconds), and marked keratoconjunctivitis sicca, assessed by fluorescein test and rose bengal staining, which were performed after impression cytology to avoid nonspecific staining of specimens.

All patients gave their informed consent for collecting impression cytology specimens and processing them by immunochemistry, and all procedures were performed in accordance with the tenets of the Declaration of Helsinki, after approval was obtained from the ethics committee of Ambroise Paré Hospital, Hôpitaux de Paris, Boulogne, France.

Conjunctival Cell Line Culture

A human conjunctival cell line (Wong–Kilbourne derivative of Chang conjunctiva, clone 1-5c-4, ATCC CCL-20.2) was cultured under standard conditions (5% CO_2, 95% humidified air, 37°C) in Eagle's minimal essential medium supplemented with 10% fetal calf serum, 2 mM L-glutamine, 50 mg/ml streptomycin, and 50 IU/ml penicillin. Cells were plated in 75-cm^2 flasks (Falcon; Becton Dickinson Labware, Plymouth, UK). They were treated with IFNγ or TNFα at least 24 hours after passage (1:4 split ratio at confluence). Human recombinant IFNγ (Pepro Tech, Rocky Hill, NJ) and TNFα (R&D Systems, Minneapolis, MN) were dissolved in culture medium at concentrations of 300 U/ml and 1100 U/ml, respectively.

Flow Cytometry

Impression cytology specimens were collected using 20 μm polyether sulfone filters (Supor membranes; Gelman Sciences, Ann Arbor, MI), as previously described.[20,21] Two pieces were applied, without exerting any pressure, onto the superior quadrants of bulbar conjunctiva, in two different areas. Care was taken to collect impression cytology only in nonexposed regions of the conjunctiva. Membranes were removed immediately after contact. They were suspended in 2 ml phosphate-buffered saline (PBS, pH 7.4) containing 0.05% paraformaldehyde until used in technical procedures. Then, they were gently agitated for 30 minutes to extract as many cells as possible without any trauma. Cells were suspended in PBS with 1% bovine serum albumin (BSA; Sigma, St Louis, MO). They were then centrifuged (1600 rpm, 5 minutes) and incubated with monoclonal antibodies.

Four antibodies were used, mouse IgG1 anti-human CD40 (clone MAB89,1 mg/ml; Immunotech, Luminy, France), mouse anti-human CD40 ligand (clone TRAP1,1 mg/ml; Immunotech), mouse IgG1 anti-HLA DR α-chain (clone TAL.1B5, 50 μg/ml; Dako, Copenhagen, Denmark), and nonimmune mouse IgG1 (Immunotech) as a negative isotypic control. Antibodies were used in 1:50 to 1:100 working dilutions in 1% BSA containing PBS, according to instructions of the manufacturers.

After 30 minutes of incubation, cell suspensions were washed in PBS by a 5-minute centrifugation and reacted with the secondary fluorescein-conjugated goat anti-mouse immunoglobulins in a 1:100 dilution for 20 minutes. They were then centrifuged in PBS (1600 rpm, 5 minutes), resuspended in 400 μl PBS, and analyzed on a flow cytometer (FACScan; Becton Dickinson, Mountain View, CA), according to a previously validated method.[22,23] The linear plot showing granulometry (side scatter) versus cell size (forward-angle light scatter) mainly revealed a single-cell population. Analytic gates were set around this population to exclude cellular debris. the number of positive conjunctival cells was then obtained from a histogram representing mean fluorescence intensities on a 4-decade logarithmic amplifier. The superior level of fluorescence intensity obtained for the isotypic control antibody was considered to be the limit of background fluorescence and the threshold of positivity for the tested antibodies. At least 2500 cells were analyzed for each marker. All specimens were analyzed in a masked manner, in that the examiners did not know the clinical history of patients.

For flow cytometric analysis of CD40 and HLA DR expressions by Chang epithelium, cells were harvested with trypsin-EDTA, pelleted, washed twice in PBS, and incubated for 30 minutes with fluorescein isothiocyanate (FITC)– conjugated anti-CD40 (clone BE-1; Pharmingen, San Diego, CA), FITC-conjugated anti-HLA DR (clone Immu-357; Immunotech), and FITC-conjugated mouse IgG1 as a negative isotypic control. CD40L labeling was performed as described earlier.

Flow Cytometric Quantitation of Fluorescence

To improve the reliability of flow cytometric analyses for a prolonged period, ex vivo measurements of fluorescent antibodies binding to conjunctival cells were quantified by translating the mean fluorescence intensities observed on fluores-

TABLE 1. Global Results of Flow Cytometry

Antibodies	Normal Eyes (n = 104)	Dry Eyes (n = 65)	Chronic Conjunctivitis (n = 69)
CD40			
% of positive cells	69 ± 24	86 ± 17*	88 ± 13*
AUF	34,164 ± 13,919	61,306 ± 28,110*	51,582 ± 19,718*
CD40L			
% of positive cells	10 ± 11	7 ± 10	14 ± 22
AUF	3,126 ± 3,010	6,766 ± 10,663	5,307 ± 5,382
HLA DR			
% of positive cells	6 ± 9%	58 ± 33*	60 ± 22*
AUF	6,679 ± 7,009	98,783 ± 102,060*	64,782 ± 80,555*

AUF, arbitrary units of fluorescence.
*$P < 0.001$ as compared to % of positive cells and AUF in normal eyes.

cence histograms into standardized arbitrary fluorescence units. A calibration curve was established by using different beads with standardized fluorescence intensities (Immunobrite; Coulter, Hialeah, FL), as previously described.[20,24] These calibrated fluorescent beads mimic different fluorescence intensities and therefore allow a reliable quantitation of fluorescence. Fluorescence levels of fluorochrome incorporated into the beads are expressed in arbitrary units of fluorescence (AUF). Fluorescence levels of CD40, CD40L, and HLA DR were calculated after converting mean fluorescence intensities into AUF units and subtracting AUF values of the background nonspecific staining given by the negative isotypic control. Statistical analysis was performed using the unpaired Student's *t*-test for between-group comparisons of mean fluorescence units and percentages of positive cells. Coefficients of correlation were also calculated at a 0.05 threshold of significance, using statistical analysis software (Statview 4; Abacus Concepts, Berkeley, CA).

The results of in vitro investigations are reported as mean intensities and represented in cytometric tracings or bar charts. Mean intensity results were calculated as arithmetic means (±SD), and significance values were calculated by means of the unpaired Student's *t*-test with $P < 0.05$ regarded as significant. All in vitro experiments were performed in duplicate.

Immunocytochemistry

In parallel, standard immunochemistry was performed in 10 normal patients, 10 specimens from the group of chronic conjunctivitis and 10 patients with dry eye, to assess morphologic patterns. Transfer of cells collected by polyether sulfone membranes to glass slides was performed immediately after cell sampling, according to a previously published method.[17,18] Indirect immunofluorescence was then performed using the same antibodies as were used for flow cytometry. Propidium iodide in a 1:500 dilution was added for 5 minutes to mark cell nuclei, before reading on an epifluorescence microscope (Leica, Deerfield, IL).

RESULTS

Ex Vivo Flow Cytometry

Global results for flow cytometry are summarized in Table 1. The number of cells in each specimen was assessed by means of a hemocytometer. The number in all specimens examined ranged between 25,000 and 200,000 cells.

Flow cytometric analysis of percentages of conjunctival cells positive to anti-CD40 antibody consistently showed the expression of CD40 in all specimens; 15% to 99% of conjunctival cells (mean 80% ± 20% of all cells) were found reacting at levels above that in the isotypic control (Figs. 1A, 1B). Significant differences could be found among the three groups of patients in percentages of positive cells (69% ± 24% in normal eyes versus 86% ± 17% and 88% ± 13%, in dry eyes and chronic conjunctivitis, respectively, $P < 0.0001$). Quantitation in standardized units of fluorescence emitted by conjunctival cells showed CD40 expression at significantly lower levels in normal (34,164 ± 13,919 AUF) than in dry eyes (61,306 ± 28,110 AUF; $P < 0.0001$), and chronic conjunctivitis (51,582 ± 19,718 AUF; $P < 0.001$).

CD40L was only weakly and inconstantly found in normal and pathologic eyes (72% of tested eyes, similarly, in the three groups). The percentage of positive cells varied from 0% to 40%. Quantitation of fluorescence found very weak fluorescence in normal eyes (3,126 ± 3,010 AUF), as well as in pathologic eyes (5,307 ± 5,382 AUF in chronic conjunctivitis, 6,766 ± 10,663 AUF in dry eyes). There was no difference among the three groups in percentages of positive cells and levels of fluorescence, which were similarly weak and variable.

Almost no HLA DR expression was found in normal eyes (Fig. 1C). Only a minority (less than 10% of all cells) expressed HLA DR at low levels of fluorescence (6,679 ± 7,009 AUF). By contrast, although clinically not or weakly inflammatory, all but six eyes with keratoconjunctivitis sicca and all eyes with chronic conjunctivitis showed high percentages of HLA DR–positive cells (16%–99% of all collected cells; mean 59% ± 27%; Fig. 1D). No difference was found between percentages of positive cells in these two groups. In fact, quantitation of HLA DR expression showed normal eyes at highly significantly lower levels compared with dry eyes (6,679 ± 7,009 AUF versus 98,783 ± 102,060 AUF; $P < 0.0001$) and chronic conjunctivitis (64,782 ± 80,555 AUF; $P < 0.0001$) with a significant difference between the two pathologic groups ($P < 0.01$).

In percentages of positive cells, HLA DR was significantly correlated with CD40 ($R^2 = 0.45$; $P < 0.0001$) and CD40L ($R^2 = 0.24$; $P < 0.02$). As expected, percentages of positive cells and AUF were highly significantly correlated for each marker ($P < 0.0001$ for HLA DR and CD40; $P = 0.0003$ for

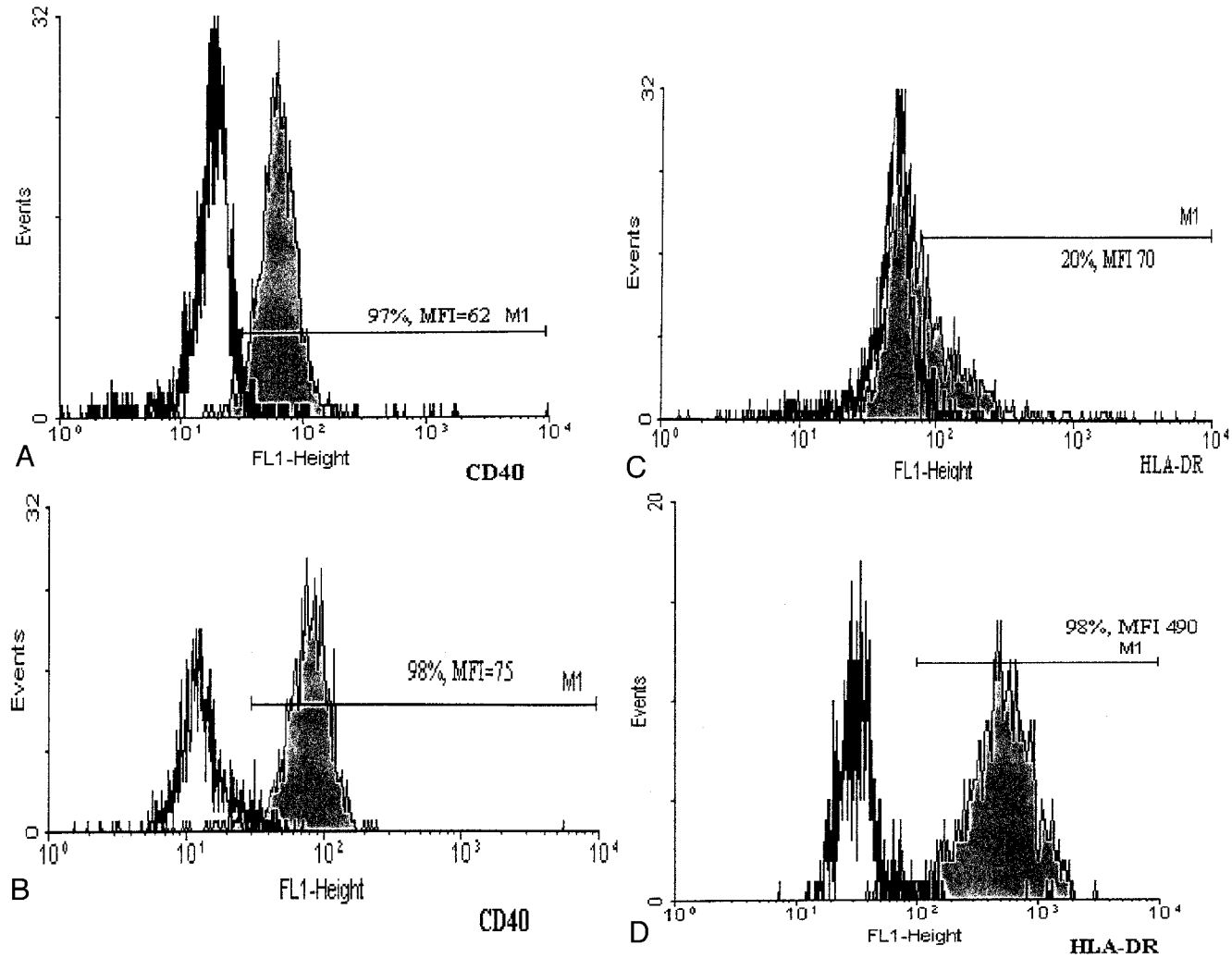

Figure 1. (**A**) Flow cytometric analyses of CD40 in impression cytology specimens. Histogram from a normal eye (97% of cells positive at a mean fluorescence intensity [MFI] of 62 = 60,740 AUF). (**B**) Histogram from a dry eye showing higher expression of CD40 by conjunctival cells (98% of cells positive at an MFI of 75 = 85,400 AUF). (**C**) Flow cytometric analyses of HLA DR in impression cytology specimens. Histogram from a normal conjunctiva showing a low HLA DR reactivity (20% of cells positive at an MFI of 70 = 18,150 AUF) of weakly positive cells on the *right side* of the control histogram. (**D**) Histogram from a dry eye showing a very high expression of HLA DR (98% of cells positive at an MFI of 490 = 253,260 AUF). All analyses were performed in at least 2500 cells. The *empty black histogram* is the negative isotypic control.

CD40L). CD40L was significantly correlated with CD40 in fluorescence ($R^2 = 0.43$, $P < 0.0001$) but not in percentages of positive cells ($R^2 = 0.15$, nonsignificant).

In Vitro Flow Cytometry

Twenty-eight percent of untreated cells expressed CD40 at a moderate level. Twenty-four hours of treatment with 300 U/ml IFNγ significantly increased CD40 expression (Fig. 2A). After 48 hours of treatment, this expression diminished, probably because of IFNγ cytotoxic action observed by contrast-phase microscopy (data not shown). TNFα (1100 U/ml) upregulated CD40 after 48 hours but not after 24 hours of exposure (Fig. 2A). No cytotoxic effect of TNFα was detected. CD40 ligand was negative in untreated cells (Fig. 2B) and its expression was not modified by TNFα or IFNγ treatments (data not shown). Untreated cells did not express HLA DR. IFNγ significantly upregulated HLA DR after 24 hours of treatment, whereas TNFα had no effect on HLA DR expression (Fig. 2C).

Immunocytology

In normal eyes, there was a weak but constant expression of CD40 (Fig. 3A) by epithelial cells but not by dendriform ones. There was no epithelial expression of HLA DR (Fig. 3B) or CD40L, whereas HLA DR was expressed at high fluorescence levels by a minority of cells with dendritic morphology.

In pathologic eyes, we observed a reliably increased density of HLA DR–positive dendriform cells in the two groups. There was an epithelial expression of HLA DR by 20% to 100% of cells in all but three specimens and a constant expression of CD40 by epithelial cells. No dendritic cell was found to be positive to anti-CD40 and CD40L antibodies in any specimen.

Discussion

In the present study, surface conjunctival epithelium normally expressed CD40 and to a lesser extent, CD40 ligand. CD40

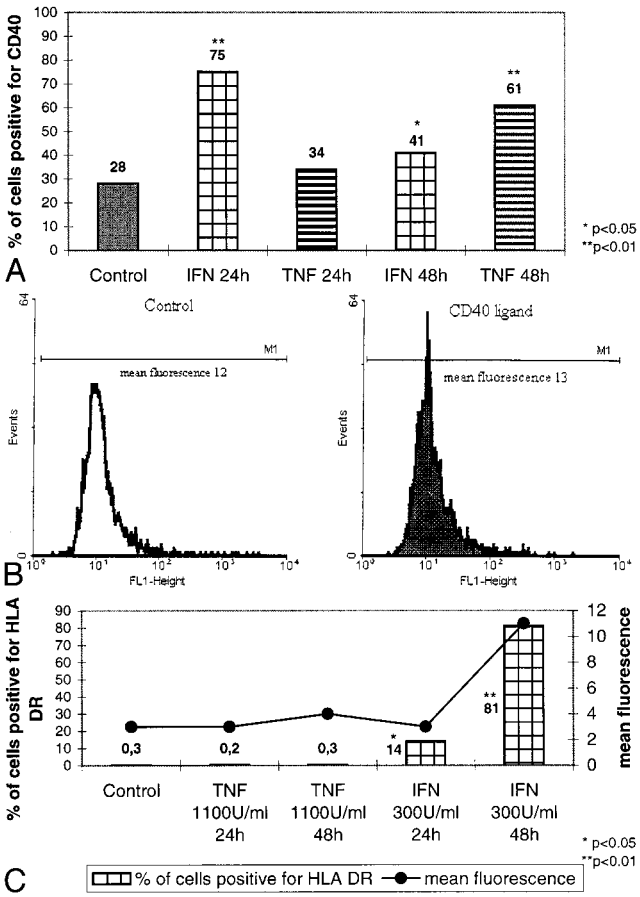

FIGURE 2. (**A**) Flow cytometric analysis of expression of CD40 antigen after IFNγ or TNFα treatments of Chang conjunctival cells. CD40 is expressed at a low level in untreated cells (28% of cells positive). A significant increase in CD40 expression is apparent respectively after 24 and 48 hours of IFNγ and TNFα treatments. (**B**) Flow cytometric analysis of CD40 ligand expression in untreated cells of Chang conjunctiva. The *empty black graphs* represent an isotypic negative control (*left*). The *gray filled graphs* represent CD40 ligand expression (*right*). There was no significant difference between control and CD40 ligand mean fluorescence, which indicates no basal expression of CD40 ligand in Chang conjunctival cells. (**C**) Flow cytometric analysis of expression of HLA DR after IFNγ or TNFα treatments of Chang conjunctival cells. Untreated cells were negative for HLA DR. There was a significant increase in HLA DR expression induced by 300 U/ml IFNγ after 24 hours of treatment. TNFα did not modify HLA DR basal expression, which remained negative.

immunostaining morphologically confirmed flow cytometric data. Interestingly, CD40 expression was significantly increased in inflammatory specimens and positively correlated with the level of expression of HLA DR antigen, chosen as a biologic standard for conjunctival inflammation. Thus, CD40 appeared to be coexpressed with HLA DR in dry eyes and in patients with chronic conjunctivitis, whereas normal eyes expressed CD40 at lower levels only.

CD40 has recently been identified on epithelial neoplasms of various locations as well as on normal epithelia. Immunohistochemical staining showed expression of CD40 on the basal (proliferating) epithelial layer of nasopharynx, tonsil, and ectocervix.[25] CD40 has also been found to be expressed in thymic[4] and thyroid epithelial cells,[6] as well as on cultured proximal tubular epithelial cells from human kidneys,[5] cultured corneal cells,[14] and retinal pigment epithelial cells.[13]

Although the expression of CD40 is generally low in normal tissues, the molecule has been found to be upregulated in various pathologic conditions including neoplasm and inflammation.[26,27] CD40 is usually expressed on cells with high proliferative potential, such as hematopoietic progenitors, B lymphocytes, and epithelial cells. In vitro activation of human B lymphocytes through CD40 induces activation and proliferation of these cells and results in the production of immunoglobulins. CD40 also interferes in B cells with other signaling pathways such as the Fas-Fas ligand pathway. CD40 cross-linking has been shown to induce Fas expression and sensitivity for Fas-mediated apoptosis.[7] Thymic epithelial cells secrete granulocyte-macrophage colony-stimulating factor after stimulation with anti-CD40 in conjunction with IL-1 and IFNγ.[4] Cross-linking of CD40 on proximal tubular epithelial cells also results in production of cytokines such as IL-6, IL-8, MCP-1, and

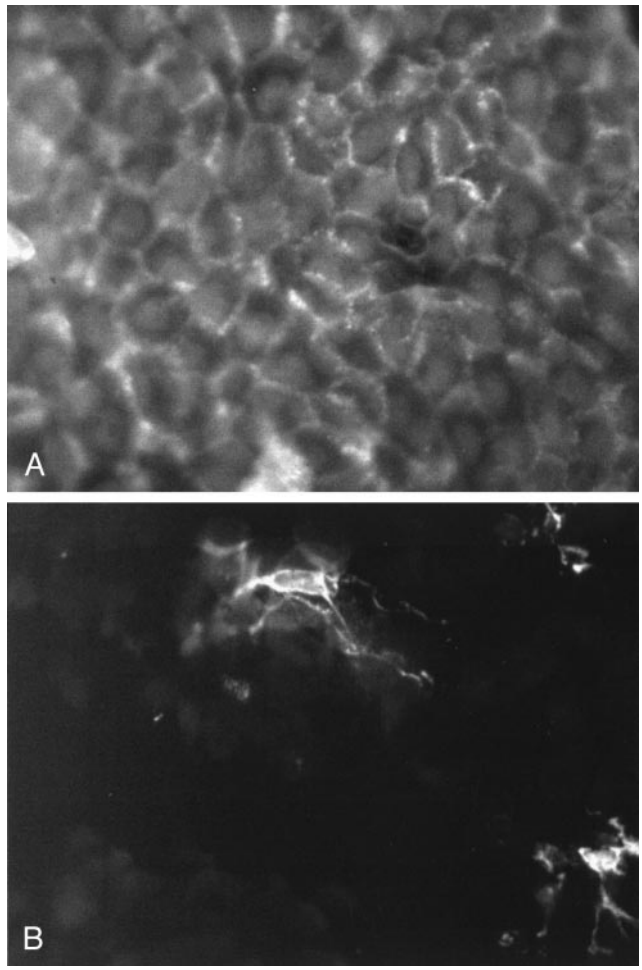

FIGURE 3. (**A**) Immunocytology in impression cytology from a patient with dry eyes: mosaic-like immunostaining of CD40 by conjunctival epithelial cells. No dendriform cells expressed CD40 (indirect immunofluorescence, weak staining of nuclei by propidium iodide). (**B**) Negative immunostaining of epithelial cell in a normal eye using anti-HLA DR antibodies. Dendriform cells were strongly positive to class II antigens (very weak staining of nuclei by propidium iodide). Magnification, ×800.

RANTES.[5] However, the exact significance and the role of CD40 expression in the ocular surface is currently unclear.

The results of the present study may suggest that conjunctival cells express HLA DR class II antigens but also costimulatory molecules such as CD40 and Fas, as previously demonstrated.[20] Fas antigen or CD95 is a cell surface protein that (similar to CD40) also belongs to the TNF-R family and mediates apoptosis when bound to Fas ligand.[28] We recently showed that the expressions of Fas antigen and apoptotic markers are significantly enhanced in inflammatory eyes and correlated to HLA DR.[20] Furthermore, in our in vitro model of Chang conjunctival cells, we showed that Fas and HLA DR are concomitantly upregulated after IFNγ treatment.[20,22] We could thus hypothesize that ocular surface inflammation both stimulates Fas expression and apoptosis and induces conjunctival cells to overexpress CD40 and class II antigens.

Moreover, inflammatory mediators IL-1α, TNFα, and IFNγ has been shown to upregulate CD40 levels on cultured thymic epithelial cells.[4] Similarly, our data showed that in a human conjunctival cell line, CD40, was upregulated after cell exposure to both IFNγ and TNFα, whereas HLA DR expression increased only after IFNγ treatment. In like manner, other recently reported data showed the absence of proper action of TNFα on HLA DR expression but a synergistic effect in enhancing IFNγ-induced upregulation of HLA DR in vitro.[23] Therefore, concomitant expression of CD40 and HLA DR in our in Chang conjunctival cells seems to be consistent with ex vivo findings and could suggest antigen-presenting potential of human conjunctival epithelium.

We could thus hypothesize that in inflammatory disorders in which lymphoid cells infiltrate the conjunctiva, there may be a direct interaction between epithelial and activated lymphoid cells and/or dendritic cells, possibly involving CD40-CD40L and Fas-Fas ligand cross-linking. Recently, CD40-CD40L has been shown to be involved in in vivo priming of $CD8^+$ cytotoxic T lymphocytes.[29] CD40 and its ligand could thereby activate the dendritic cells to allow, by an indirect mechanism, T lymphocyte priming.

We found a very inconstant presence of CD40L. This could be explained by a low density of expression of these receptors on conjunctival cells or by the fact that CD40L in its soluble form may be more predominant than the membrane-bound form and therefore would not be detected in impression cytology specimens. Other molecules have indeed been reported to be alternative ligands for CD40, providing similar signals to activated cells.[2] CD40 may in fact bind this natural ligand (CD40L) as well as other members of the TNF-R superfamily. Moreover, the absence of CD40L in vitro and its inconstant detection ex vivo, although significantly correlated with CD40 and HLA DR, is in agreement with formerly reported data concerning skin expression of CD40L present only in some IgE-associated diseases such as atopic dermatitis and scabies.[30] In these diseases, epithelial CD40L has been suggested to contribute to IgE synthesis. Similarly, we recently reported IgE secretion enhancement in chronic conjunctival impairment (inflammation, dry eye) even without any clinical evidence of allergy.[31] It could similarly be hypothesized that CD40L expression may be associated with IgE secretion in some conjunctival inflammatory diseases. The absence of effect of IFNγ and TNFα on CD40L synthesis would indicate that by contrast with CD40, CD40L is not under the influence of cytokines of the T helper 1 system. Further studies are necessary to investigate the expression of CD40 and CD40L in allergic ocular surface disorders and to determine whether CD40L would provide a useful indicator for IgE-mediated diseases.

In conclusion, in this study conjunctival epithelial cells normally expressed CD40 antigen and, more inconsistently, its ligand. CD40 expression was upregulated in inflammatory eyes and was positively correlated with HLA DR expression. We could thus hypothesize that CD40 may be an important molecule in both the cellular and humoral immune responses of the human conjunctiva. The exact mechanisms and functional meaning of this interaction between CD40 and HLA DR as well as between apoptotic and inflammatory pathways in ocular surface disorders have yet to be made clear.

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