EXTENDED REPORT

Ocular surface epithelium induces expression of human mucosal lymphocyte antigen (HML-1) on peripheral blood lymphocytes

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Background/aims: Peripheral blood CD8+ lymphocytes that home to mucosal surfaces express the human mucosal lymphocyte antigen (HML-1). At mucosal surfaces, including the ocular surface, only intraepithelial CD8+ lymphocytes express HML-1. These lymphocytes are retained in the intraepithelial compartment by virtue of the interaction between HML-1 and its natural ligand, E-cadherin, which is expressed on epithelial cells. The purpose of this study was to determine whether ocular surface epithelial cells (ocular mucosa) could induce the expression of human mucosal lymphocyte antigen on peripheral blood lymphocytes.

Methods: Human corneal and conjunctival epithelial cells were co-cultured with peripheral blood lymphocytes. Both non-activated and activated lymphocytes were used in the experiments. After 7 days of incubation, lymphocytes were recovered and analysed for the antigens CD8/HML-1, CD4/HML-1, CD3/CD8, CD3/CD4, CD3/CD25, CD8/CD25, and CD4/CD25 by flowcytometry.

Results: Significant statistical differences were observed in the CD8/HML-1 expression when conjunctival epithelial cells were co-cultured with non-activated and activated lymphocytes ($p = 0.04$ for each) and when corneal epithelial cells were co-cultured with non-activated lymphocytes ($p = 0.03$). Significant statistical difference in CD4/HML-1 expression was observed only when conjunctival epithelial cells were co-cultured with activated lymphocytes ($p = 0.02$).

Conclusion: Ocular surface epithelial cells can induce the expression of human mucosal lymphocyte antigen on CD8+ (and to some extent on CD4+) lymphocytes. This may allow the retention of CD8+ and CD4+ lymphocytes within the epithelial compartment of the conjunctiva and play a part in mucosal homing of lymphocytes.

The mucosal immune system traditionally includes mucosa associated lymphoid tissue (MALT) of the gastrointestinal, respiratory, and urogenital tract.[1–5] Intraepithelial lymphocytes (IELs) of mucosal epithelia are predominantly CD8+ while the lamina propria shows equal amounts of CD8+ and CD4+ lymphocytes.[3] IELs express the human mucosal lymphocyte antigen (HML-1), and show preferential homing to mucosal surfaces.[2] The IELs are a specialised subpopulation of T cells, which intercalate between the epithelial cells of the mucosal epithelium.[3] In addition to co-expressing predominantly CD8 and HML-1 antigens, the majority of them in humans are T cell receptor αβ positive.[3] Functionally, it is believed that these specific lymphocytes may have a special role in immunological defence by means of cell mediated cytotoxicity,[3] and perhaps also in tolerance mechanisms that operate at mucosal surfaces.[3 6–10]

The HML-1 antigen is an αEβ7 integrin expressed on approximately 95% of intraepithelial CD8+ lymphocytes, but only on 1%–2% of peripheral blood lymphocytes.[3 11–16] Its expression can be increased after stimulation of lymphocytes with mitogen or culturing with TGFβ-1.[11 15 17–19] It mediates specific adhesive interactions between intraepithelial lymphocytes and a tissue restricted adhesion molecule on mucosal epithelial cells, called E-cadherin.[15 19] HML-1 integrin may also mediate a co-stimulatory signal for lymphocyte activation.[17]

There is substantial evidence to indicate that the conjunctival mucosa (conjunctiva associated lymphoid tissue, CALT) is also part of the mucosal immune system.[2 9 20–25] Although lymphoid aggregates are not widely found in normal conjunctiva, follicles of lymphoid cells are a characteristic feature of several chronic inflammatory conjunctival diseases. Recently, high endothelial venules expressing lymphocyte homing receptors have also been demonstrated in normal human conjunctiva.[26] In a previous study, we were able to identify the presence of the CD8+/HML-1+ lymphocytes in the conjunctival epithelium, lacrimal gland, and corneoscleral limbus in human cadaver eyes and in specimens of conjunctival intraepithelial neoplasia.[2 27] The ligand E-cadherin has also been demonstrated on conjunctival epithelial cells.[28]

In this study we investigated the induction of HML-1 expression on lymphocytes by ocular surface epithelial cells.

METHODS

Purified peripheral blood lymphocytes (activated and non-activated) were co-cultured with primary cultures of human conjunctival and corneal epithelial cells and examined for expression of HML-1 antigen.

Experimental design

Lymphocytes obtained from the peripheral blood of healthy patients were diluted to 1×10^6 cell/ml. A volume of 100 µl of

Abbreviations: CALT, conjunctiva associated lymphoid tissue; HML-1, human mucosal lymphocyte antigen; IELs, intraepithelial lymphocytes; MALT, mucosa associated lymphoid tissue; TGF-β, transforming growth factor beta

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each sample was analysed by flowcytometry to determine the initial phenotypic profile (CD8+, CD4+, CD25+) of the lymphocytes.

The lymphocytes (1 ml of 1×10^6 cells per ml) were co-cultured with confluent epithelial cell cultures wherein (a) the lymphocytes were in direct contact with the epithelial cells (group A) and (b) the lymphocytes were separated from the epithelial cells by a 0.45 μm pore size hydrophilic cyclopore semipermeable membrane of polyethylene terephthalate (Falcon 25 mm cell culture insert, Becton Dickinson Company, Franklin Lakes, NJ, USA), which prevents direct contact between cells but allows free interchange of culture media between the epithelial cell and lymphocyte compartments (group B). Lymphocytes were also maintained in a culture well with medium alone (control, group C). On day 7, the lymphocytes were recovered[29] suspended in 2 ml of culture medium and analysed by flowcytometry.

The above experiment was performed with conjunctival and corneal epithelial cells using non-activated and activated lymphocytes. The epithelial nature of the cultured corneal and conjunctival cells was confirmed by staining with specific antibodies against cytokeratin 3 and 19 and against fibroblasts (to rule out excessive contamination with these cells). HML-1 is a very late activation antigen. Its expression is increased in activated lymphocytes. It was therefore important to determine whether expression of this antigen is related to ''contact'' with epithelial cells or to the state of activation of lymphocytes. Hence both activated and non-activated lymphocytes were examined.

Corneal limbal and conjunctival epithelial cell culture

Primary cultures of human corneal limbal epithelial cells were prepared as described by Ebato *et al*.[30] Briefly, 15 human corneoscleral rims from cadaver eye donors were used. Each rim was divided into six explants (2 mm each) and placed epithelial side up in each well of a Falcon Primaria 35 mm tissue culture plate (Becton Dickinson and Company, Franklin Lakes, NJ, USA). Serum free lymphocyte medium AIM-V (Life Technologies, Inc, Grand Island, NY, USA) enriched with 5 μg/ml of crystalline bovine insulin (Life Technologies, Inc. Chagrin Falls, OH, USA) and 0.01 μg/ml of human recombinant epidermal growth factor (Life Technologies, Inc, Grand Island, NY, USA) was used in the cultures.[31] A volume of 3 ml of medium were added to each well and the medium was changed twice a week for 2 weeks. Explants were left in the culture dish for the duration of the incubation.

For conjunctival epithelial culture, 11 biopsy specimens (less than 10 mm^2) of clinically normal tissue were obtained from 11 patients who underwent conjunctival autograft for pterygium surgery (n = 1), enucleation for choroidal melanoma (n = 2), conjunctival tumour resection (n = 5), brachytherapy for iris melanoma (n = 1), and oculoplastic procedures that involved conjunctival manipulation (n = 2). These specimens were obtained in accordance with the tenets of the Declaration of Helsinki.[32] Under sterile conditions, the tissue was cut into six explants (1 mm each). These were placed epithelial side up in each well of a Falcon Primaria 35 mm tissue culture plate (Becton Dickinson) and maintained as described above for corneal limbal cultures. The epithelial cell characteristics of the cultured cells was confirmed in all samples by their cobblestone pattern under phase contrast microscopy evaluation and by immunohistochemistry of confluent epithelial sheets and indirect flowcytometry using the following antibodies: AE-5, a mouse monoclonal antibody specific for cytokeratin 3 and cytokeratin 19 (ICN Biomedicals, Inc, Costa Mesa, CA, USA); and a mouse anti-human fibroblast (Dako Corporation, Carpinteria, CA, USA).[33 34]

Lymphocytes separation and activation

A volume of 5–10 ml of peripheral venous blood was obtained from normal volunteers, diluted 1:2 with Dulbecco's phosphate buffered saline (D-PBS; Life Technologies Inc, Grand Island, NY, USA) and separated by centrifugation at $400\times g$ for 30 minutes on Ficoll/Hypaque ET (Pharmacia Fine Chemicals, Uppsala, Sweden) density gradient to obtain isolated mononuclear cells.[35] The cells were washed in D-PBS, centrifuged at $100\times g$ for 7 minutes, and washed again in culture medium. Cells were counted in a haematocytometer and tested for viability using trypan blue dye exclusion.[36] The mononuclear cells were then placed in the same medium used for the epithelial cell cultures.

For the in vitro activation experiments, lymphocytes were incubated with 2.5 ng/ml of phorbol 12-myristate 13-acetatein (PMA, Sigma Chemical Inc, St Louis, MO, USA) and 250 ng/ml of Ionomycin (Sigma Chemical Inc, St Louis, MO, USA) at 37℃ for 5 hours. After stimulation, the cells were washed three times with culture medium and plated in 24 well 16.2 mm diameter cell culture cluster (Costar Corporation, Cambridge, MA, USA) until use in the experiment (on the same day).[37] Besides being used in co-culture experiments with epithelial cells, activated lymphocytes were also directly stained with antibodies against CD3, CD4, CD8, CD25, and HML-1 antigens (see below under flowcytometry) on days 1 and 7 after activation.

Flowcytometry

Lymphocytes were stained with saturating concentrations of combinations of directly conjugated monoclonal antibodies: anti-Leu-4/FITC and anti-Leu-4/PE (CD3), anti-IL-2R/FITC (CD25) (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA), anti-Leu-2a/PE (CD8), and anti-Leu-3a/PE (CD4); and indirectly FITC conjugated monoclonal antibodies: Monoclonal mouse anti-human mucosal lymphocyte antigen (HML-1, Ber-ACT8) (Dako, Glostrup, Denmark) and goat anti-mouse Ig FITC (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA), to produce the following pairs of antibodies: CD3/CD4, CD3/CD8, CD3/CD25, CD4/CD25, CD8/CD25, CD4/HML-1, CD8/HML-1. Two controls were used: isotype matched IgG mouse Mb conjugated to FITC/PE not reactive with human leucocytes to determine non-specific fluorescence; and the CD45/CD14 antibody as a gating control for lymphocytes. Both lymphocytes and epithelial cells were also stained with MHC class I and class II antibodies (Anti-human MHC class I and anti-human HLA-DP, DQ, DR antigen, Becton Dickinson Immunocytometry Systems, San Jose, CA, USA). After staining, cells were fixed for 30 minutes with 1% paraformaldehyde, and 1×10^4 cells were analysed by the dual laser flowcytometry FACScan (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA) and Epics (Epics cell sorter, Coulter Electronics Inc, Hialeah, FL, USA), using FACScan and Consort-30 software programs provided by Becton Dickinson. Lymphocyte populations were gated by forward/sideward scatter analysis to exclude monocytes, epithelial cells, and dead cells.[38 39] At least 10^4 cells were counted and the positive cells expressed as a percentage of the total cells counted.

After completion of the experiment, four conjunctival and four corneal epithelial cultures were removed from the bottom of the well with a cell spatula and examined for the presence of lymphocytes. This was to ensure that the results obtained from the various experiments were not influenced by a difference in residual lymphocytes remaining attached to the epithelial cultures. There was no statistical difference in

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the number of residual lymphocytes remaining in samples thus obtained.

Statistical analysis

The Kolmogorov-Smirnov test was performed to evaluate the distribution in each experimental condition and the null hypothesis of normality was not rejected.

The statistical analysis used to compare groups A, B, and C for antigen expression was performed in two steps:

(1) Repeated measures analysis of variance (ANOVA) to compare the three different groups overall (for example, CD8+/HML-1+ from group A *v*. CD8+/HML-1+ from group B *v* CD8+/HML-1+ from group C).

(2) For any result from step 1 that was significant, paired *t* tests were used for post hoc comparisons (for example, CD8+/HML-1+ from group A *v* CD8+/HML-1+ from group B, CD8+/HML-1+ from group B *v* CD8+/HML-1+ from group C, CD8+/HML-1+ from group A *v* CD8+/HML-1+ from group C).

The same type of statistical analysis was performed for comparing antigen expression before activation, 1 day after activation, and 7 days after activation. All statistical tests were performed at the 5% probability level.

RESULTS

Using flowcytometry we compared lymphocyte antigen expression among groups A (lymphocytes in direct contact with epithelial cells), B (lymphocytes separated from epithelial cells by a 0.45 µm membrane), and C (culture medium and lymphocytes), using both non-activated and activated lymphocytes, after 7 days of incubation.

None of the lymphocyte antibodies used showed presence of lymphocytes in the negative control comprising corneal and conjunctival epithelial cells—that is, there were no lymphocytes from the limbal explants appearing in the culture.

CD8/HML-1

Groups A, B, and C were significantly different (ANOVA) for CD8+/HML-1+ expression in all experiments except when corneal epithelial cells were co-cultured with activated lymphocytes (fig 1, table 1).

The post hoc multiple comparisons showed that when corneal epithelial cells were co-cultured with non-activated lymphocytes, groups A and B had significantly more CD8+/HML-1+ lymphocytes than group C ($p = 0.04$ and $p = 0.05$, respectively). No statistically significant difference ($p = 0.08$) could be detected between the different groups when corneal epithelial cells were co-cultured with activated lymphocytes. When conjunctival epithelial cells were co-cultured with non-activated lymphocytes, group A had significantly more CD8+/HML-1+ lymphocytes than the control group C ($p = 0.047$). When conjunctival epithelial cells were co-cultured with activated lymphocytes, group A had significantly more CD8+/HML-1+ lymphocytes than group B ($p = 0.02$) and group C ($p = 0.033$). Although only a small percentage of lymphocytes express HML-1 after exposure to epithelial cells, the fluorescence intensity of these cells was very high suggesting that HML-1 expression was substantial (fig 2).

CD4/HML-1

For CD4+/HML-1+ expression, groups A, B, and C (ANOVA) were significantly different only in the experiment where conjunctival epithelial cells were co-cultured with activated lymphocytes ($p = 0.02$). The post hoc multiple comparisons showed that group A had significantly more CD4+/HML-1+ lymphocytes than group B ($p = 0.021$) and group C ($p = 0.047$) (fig 3, table 2). CD4+ lymphocytes expressing HML-1 after exposure to corneal cells are very few. The intensity of expression in CD4+/HML-1 positive cells was high (fig 4).

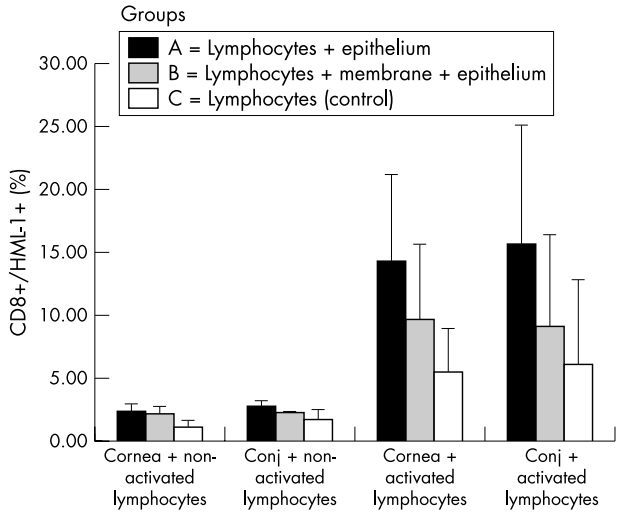

Figure 1 Means and standard deviations of CD8+/HML-1+ lymphocytes expressed as a percentage of the total gated lymphocyte population for groups A, B, and C in the different experimental conditions. Number of samples: Cornea + non-activated lymphocytes; A = 4; B = 4; C = 4 Conj + non-activated lymphocytes; A = 3; B = 3; C = 3; Cornea + activated lymphocytes; A = 4; B = 4; C = 3; Conj + activated lymphocytes; A = 6; B = 6; C = 5.

CD3/CD8, CD3/CD4, CD3/CD25, CD8/CD25, CD4/CD25, MHC

There were no significant differences among the three groups in all experiments with regard to CD3/CD8, CD3/CD4, CD3/CD25, CD8/CD25, CD4/CD25 expression—that is, the distribution of the different cell types was uniform in all four groups and thus did not introduce a bias in any of the results mentioned above with regard to HML-1 antigen expression (data not shown). Ten per cent of epithelial cells in culture expressed class II MHC. This number did not show any increase when cells were co-cultured with activated or non-activated lymphocytes.

Lymphocyte activation (fig 5)

A statistically significant difference was observed in the comparison among the three different time points at which expression of HML-1 on CD8+ lymphocytes was examined

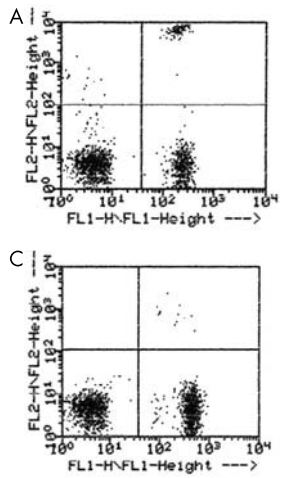

Figure 2 Cells were cultured, harvested, and stained for CD8 and HML-1 as described in the methods. Flowcytometry dot plots for groups A and C are shown. There is significant increase in fluorescence intensity in the positive cells (data presented is from one significant experiment out of eight).

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Table 1 Statistical analysis of CD8/HML-1 expression in groups A, B, and C for the different experimental conditions

Condition	A *v* B *v* C (p)	A *v* B	Multiple comparison B *v* C	A *v* C
1 Cornea+ non-activated cells	0.03*	–	*	*
2 Conjunctiva + non-activated cells	0.04*	–	–	*
3 Cornea+ activated cells	0.08	–	–	–
4 Conjunctiva + activated cells	0.04*	*	–	*

A = lymphocytes co-cultured with epithelial cells; B = lymphocytes co-cultured with epithelial cells but separated from them with a semipermeable 0.45 μm membrane; C = epithelial cells in culture medium alone (control group); A *v* B *v* C, results of repeated measures ANOVA; *p<0.05.

(p = 0.0061 ANOVA). A progressive increase in expression was observed from day 0 (before activation) to day 7.

With regard to HML-1 expression on CD4+ lymphocytes, there was no significant difference between days 0, 1 and 7 of activation (p = 0.9259 ANOVA).

Expression of CD25 was significantly increased on CD3, CD8 and CD4 lymphocytes after activation (p = 0.0001, p = 0.0003, and p = 0.0002). The increase of CD25 expression was maximal on day 7 of incubation, reaching to approximately 90% of CD3+ lymphocytes. This high percentage of CD25 expression on the lymphocytes is indicative of the efficacy of the activation procedure used.

DISCUSSION

The expression of surface antigens on lymphocytes in a tissue depends on lymphocyte activation and direct cell to cell or indirect cellular interactions.[40] Each antigen thus expressed, responds quantitatively and qualitatively to a specific equation involving these factors.

Experimental models involving co-cultures or simultaneous cultures of lymphocytes and other cells have been used to study such relations. Iwata *et al*[41 42] and Shams *et al*[43] used lymphocytes from peripheral blood co-cultured with corneal epithelial cells to determine the role of epithelium and allogenic lymphocyte interaction on the lymphocytic activation process.

In the present study we examined the changes in the expression of HML-1 on peripheral lymphocytes, induced by direct and indirect interactions with ocular surface epithelium, both corneal and conjunctival, maintained in culture. The 7 day incubation period was determined based on previous findings by Schieferdecker *et al*,[18] who found that the maximum HML-1 expression on activated lymphocytes occurred between 5 and 7 days.

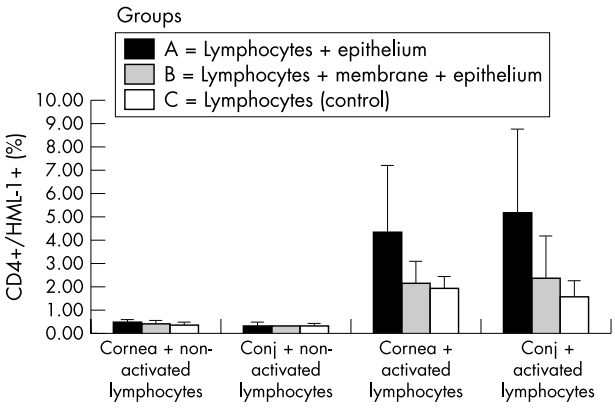

Figure 3 Means and standard deviations of CD4+/HML-1+ lymphocytes expressed as a percentage of the total gated lymphocyte population for groups A, B, and C in the different experimental conditions. Number of samples: Cornea + non-activated lymphocytes; A = 3; B = 3; C = 3 Conj + non-activated lymphocytes; A = 3; B = 3; C = 3; Cornea + activated lymphocytes; A = 4; B = 4; C = 4; Conj + activated lymphocytes; A = 6; B = 6; C = 6.

The allogeneic model does not reproduce identical conditions found by peripheral lymphocytes during recirculation through CALT. The interactions of the epithelial cells with allogeneic lymphocytes could have induced the expression of class II MHC on epithelial surface. This interaction might have promoted lymphocyte activation and increase of HML-1 expression, a typical activation antigen. However, Iwata *et al*[41] showed that expression of MHC class II on corneal epithelial cells and allogeneic lymphocytic proliferation could only be induced by addition of interferon-γ, which was not used in our experiment. Any allogeneic response caused by antigen presentation, occurring in the experiment where non-activated lymphocytes were used, would have resulted in a difference with regard to CD3/CD25, CD4/CD25 and CD8/CD25, between the control group (C) and groups A and B. In a similar pilot study, Gomes *et al*[44] studied the changes induced on surface antigens of non-activated lymphocytes from the same donor co-cultured with corneal epithelial cells from different donors. The authors observed results similar to those found in the present experiment indicating that use of allogeneic lymphocytes and epithelial cells did not particularly influence the results of the study. We therefore believe that the use of lymphocytes and allogeneic epithelial cells in this experiment is unlikely to have influenced the results.

Under normal conditions, the intestinal and conjunctival intraepithelial and lamina propria lymphocytes express more HML-1 antigen (>90% intra epithelial and 30–40% in lamina propria) than peripheral lymphocytes (1–2%).[2 3 11 14] Schieferdecker *et al*[18] described an increase of HML-1 expression on peripheral lymphocytes after stimulation with PHA, concanavalin A, IL-2 receptor, and other mitogens. However, the same findings were not observed in intraepithelial and lamina propria lymphocytes.[15 18 40] Kelleher *et al*[15] found similar results using a combined stimulation with PHA and PMA. These authors suggest that the integrin HML-1 is in fact an activation antigen, which is already fully expressed on lymphocytes of the intestinal mucosa.

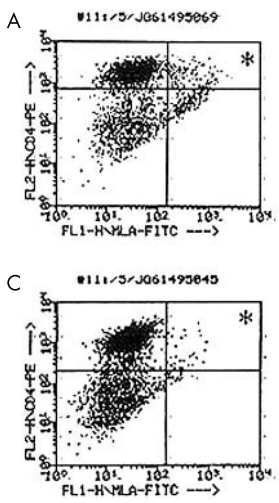

Figure 4 Cells were cultured, harvested and stained for CD4 and HML-1 as described in the methods. Flowcytometry dot plots for groups A and C are shown. There is significant increase in fluorescence intensity in the positive cells (data presented is from one significant experiment out of eight).

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Table 2 Statistical analysis of CD4/HML-1 expression in groups A, B, and C for the different experimental conditions

Condition	A v B v C (p)	Multiple comparison A v B	B v C	A v C
1 Cornea+ activated cells	0.50	–	–	–
2 Conjunctiva+ non-activated. cells	0.97	–	–	–
3 Cornea+ activated cells	0.13	–	–	–
4 Conjunctiva + activated cells	0.02*	*	–	*

A = lymphocytes co-cultured with epithelial cells; B = lymphocytes co-cultured with epithelial cells but separated from them with a semipermeable 0.45 μm membrane; C = epithelial cells in culture medium alone (control group); A v B v C, results of repeated measures ANOVA; *p<0.05.

More recently, Brew *et al*[45] showed that the HML-1 protein from peripheral lymphocytes, stimulated with PMA, is identical to the one found on IELs. These authors questioned the specificity of HML-1 on IELs and suggested that its expression may occur on peripheral lymphocytes, especially CD8+, after a specific stimulation.

To study the significance of lymphocytic activation on HML-1 expression, we performed experiments with peripheral non-activated and activated lymphocytes. Activation of lymphocytes was achieved by the combined use of PMA and ionomycin. This is considered to be a longer, more intense stimulation that does not depend on the presence of antigen presenting cells.

In the experiments with non-activated lymphocytes, HML-1 expression on CD8+ lymphocytes was found maximally in the groups co-cultured with epithelial cells for 7 days. This increase was greatest in group A, where the epithelial cells and lymphocytes were in direct contact. The same was not observed with CD4+ lymphocytes, which indicates that epithelial cells were important in HML-1 expression specifically on CD8+ lymphocytes. Importantly, no difference in expression of the activation marker, CD25, was observed between CD8 or CD4 lymphocytes in these groups, indicating that the observed difference in HML-1 expression was independent of the state of activation of the lymphocytes.

Activation by PMA and ionomycin also revealed a similar pattern with regard to HML-1 expression. Although CD25 expression progressively increased, reaching around 90% of the CD3+ cells after 7 days of incubation, a significant increase of HML-1 expression was observed on CD8+, but not on CD4+ lymphocytes. This result suggests that the HML-1 antigen, as an activation antigen, is more specific for CD8+ lymphocytes. It is conceivable that the increase in HML-1 is secondary to an increase in the expression of MHC class II molecules. It is known that allogeneic systems such as the ones we used tend to induce the expression of MHC molecules on both target cells and lymphocytes. We stained both types of cells for MHC class I and II at the same time that staining for CD4, CD8, and HML-1 were performed and no significant changes were observed.

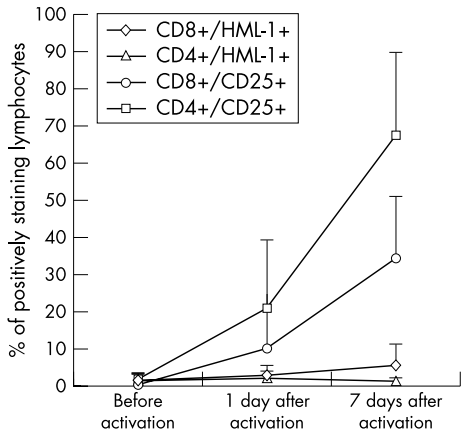

Figure 5 Means and standard deviations of positively stained lymphocyte subpopulations before activation, and on days 1 and 7 after activation. This graph illustrates that HML-1 expression was not a direct consequence of lymphocyte activation.

An increase of HML-1 expression was also observed on the lymphocytes co-cultured with epithelial cells but separated from the latter by a semipermeable membrane. This finding suggests that soluble factors produced by the epithelial cells may contribute to HML-1 expression. However, HML-1 expression was maximal in group A (direct contact of lymphocytes with epithelium, for activated and non-activated lymphocytes) and demonstrates that HML-1 expression may depend more on direct contact of lymphocytes with epithelial cells. It is also possible that a soluble product of epithelial cells would be most concentrated in the immediate vicinity of epithelial cells and might alone account for the higher HML-1 expression on cultures without the membrane.

The type of epithelium did not appear to be important as the expression of the different antigens studied was almost the same for corneal and conjunctival epithelium. Cerf-Bensussan *et al*[46] have found HML-1+ lymphocytes in bronchial and mammary epithelium, and even in the epidermis of cutaneous lesions such as parapsoriasis and graft versus host disease.

Parker *et al*[11] and Russell *et al*[17] have observed an increase of HML-1 expression on peripheral lymphocytes cultivated with transforming growth factor beta (TGF-β1). TGF-β1 is produced by different epithelial cells, including corneal and conjunctival epithelium.[47 48] This would suggests that HML-1 expression, predominantly in MALT, may be related to TGF-β1 production by the epithelium.

Intraepithelial lymphocytes are unique to mucosal surfaces where they possibly have a role in mucosal defence and tolerance mechanisms. This study illustrates that the high proportion of intraepithelial CD8+ cells expressing HML-1 is determined by an interaction between the lymphocytes and epithelial cells, possibly via a soluble factor which may require close proximity of the cells. Such an occurrence would facilitate retention of these lymphocytes in the intraepithelial compartment mediated by binding of HML-1 to E-cadherin.

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