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Accessory cell function of a human colonic epithelial cell line HT-29 for bacterial superantigens

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SUMMARY

The expression and up-regulation of cell adhesion molecules on a human colonic epithelial cell line HT-29, and the peripheral blood T lymphocyte proliferation responses to bacterial superantigens presented by this cell line were investigated, compared with peripheral blood monocytes. In HT-29 cells, there was constitutive expression of intercellular adhesion molecule-1 (ICAM-1) and lymphocyte function-associated antigen-3 (LFA-3) at a low level, but no constitutive expression of HLA-DR, LFA-1, B7-1 and B7-2 molecules. After stimulation with the supernatants of staphylococcal enterotoxin B (SEB)-stimulated peripheral blood mononuclear cells for 48 h, there was significant up-regulation of HLA-DR and ICAM-1 molecules (both > 90% positive). However, this stimulation had no effect on the expression of LFA-1, B7-1, B7-2 and LFA-3 molecules. In the presence of all tested superantigens SEB, toxic shock syndrome toxin-1, and streptococcal pyogenic exotoxin A, stimulated HT-29 cells caused significant T cell proliferation. When monocytes were used as antigen-presenting cells (APC), the MoAbs against HLA-DR, B7-2 and LFA-3 showed a significant inhibition of SEB-induced T cell proliferation. Anti-ICAM-1 MoAb had no effect on this response. On the other hand, when stimulated HT-29 cells were used as APC, the MoAbs against HLA-DR and ICAM-1 significantly inhibited SEB-induced T cell proliferation. In contrast to monocytes, anti-B7-2 and anti-LFA-3 had no effect on this response. SEB could not induce HT-29 cells to produce IL-8 directly; however, SEB significantly induced the stimulated HT-29 cells to produce IL-8 in the presence of T cells. Thus these data demonstrate that the products of superantigen-stimulated T cell activation can increase the expression of HLA-DR and ICAM-1 molecules on HT-29 cells significantly. Stimulated HT-29 cells can serve as APC to bacterial superantigens. This response is an HLA-DR- and ICAM-1-dependent, but B7-2- and LFA-3-independent process, which was different from professional APC monocytes.

Keywords intestinal epithelial cell superantigen antigen presentation costimulatory molecule IL-8

INTRODUCTION

Representative bacterial superantigens are mainly produced by *Staphylococcus aureus* and *Streptococcus pyogenes* [1,2]. Compared with conventional antigens, bacterial superantigens do not require processing by antigen-presenting cells (APC), bind to the external domain of the MHC class II molecules rather than the antigen-binding groove, and bind to the variable region of the β-chain (Vβ) of T cell receptor (TCR). Thus, superantigens activate all responder T cells expressing specific Vβ. Bacterial superantigens can be responsible for acute diarrhoeal illness in food poisoning and toxic shock syndrome in humans [1,2]. They have also attracted much attention because they have been implicated in the pathogenesis of some autoimmune diseases such as Kawasaki disease, rheumatoid arthritis, psoriasis, and diabetes mellitus [1–3]. Posnett *et al.* found that V$\beta 8^+$ T cells were elevated in the peripheral blood and mesenteric lymph nodes of a subset of Crohn's disease (CD) patients [4], suggesting a possible role for superantigens in chronic inflammatory bowel diseases (IBD) such as CD [4–6].

Intestinal epithelial cells (IEC) are the first barrier between potentially pathologic luminal microorganisms and intestinal mucosa. IEC are capable of processing and presenting protein antigens to primed T cells and induce T cell proliferation *in vitro* [7,8], and can express abundant HLA-DR molecules in the setting of inflammation such as IBD [9,10]. Human IEC cell lines can also be induced to express HLA-DR molecules after interferon-gamma

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(IFN-γ) stimulation [8,11]. Recently, growing evidence has shown that IEC can produce a variety of cytokines or chemokines after stimulation with proinflammatory cytokines, bacterial invasion, or during inflammation [12–14]. These findings suggest that IEC play an important role as active participants in the immunoregulation of intestinal mucosa. IEC are exposed *in vivo* to a wide variety of bacterial products including bacterial superantigens that pass through the intestinal tract, and these cells express increased MHC class II molecules during inflammation, therefore they are likely to serve as APC for bacterial superantigens.

In superantigen-induced T cell activation, in addition to MHC class II molecules, the ligation of costimulatory molecules CD28, lymphocyte function-associated antigen-1 (LFA-1) and CD2 expressed on T cells, and their ligands B7-1 (CD80)/B7-2 (CD86), intercellular adhesion molecule-1 (ICAM-1) and LFA-3 on APC have recently been shown to provide costimulatory function and increase T cell activation and proliferation [15–17]. IEC cell lines can be induced to express ICAM-1 molecules by IFN-γ [18,19]. Knowledge of B7-1, B7-2 and LFA-3 expression and regulation on IEC is very limited. Whether these costimulatory molecules are involved in superantigen presentation remains unknown. On the other hand, IEC can produce IL-8, a potent neutrophil chemoattractant, by cytokine stimulation or bacterial invasion [12,14], suggesting that IEC are implicated in inflammatory cell recruitment to inflammatory sites. Whether superantigens can stimulate IEC to produce IL-8 remains to be elucidated.

With a well characterized human colonic epithelial cell line HT-29 as a model of IEC, in comparison with professional APC monocytes, we have investigated (i) the expression and up-regulation of costimulatory molecules B7-1, B7-2, ICAM-1, LFA-1, LFA-3 as well as HLA-DR molecules on HT-29 cells by the supernatants of staphylococcal enterotoxin B (SEB)-stimulated peripheral blood mononuclear cells (PBMC); (ii) the capacity of this cell line to present bacterial superantigens to highly purified peripheral blood T lymphocytes and costimulatory molecules involved in this response; (iii) IL-8 production of HT-29 cells stimulated by bacterial superantigen.

MATERIALS AND METHODS

Epithelial cell lines and cell culture

Human HT-29 colonic epithelial cells (ATCC HTB38) were obtained from the American Type Culture Collection (Rockville, MD). HT-29 cells were grown in MaCoy's 5A medium supplemented with 10% heat-inactivated fetal calf serum (FCS), 2 mM glutamine and antibiotics in a humidified atmosphere of 5% CO_2. Subculturing was performed with 0·1% trypsin and 0·5 mM EDTA solution. The medium and related reagents were obtained from GIBCO (Grand Island, NY). HT-29 is a line of moderately well differentiated cells derived from a human colon adenocarcinoma. This cell line was selected as a model of IEC because it manifests important functional and morphological characteristics of normal human IEC [20].

Isolation of peripheral blood T cells and monocytes

PBMC were isolated from heparinized venous blood by Ficoll–Hypaque (Pharmacia, Uppsala, Sweden) density gradient centrifugation. Cells were washed three times with PBS and then resuspended in culture medium (RPMI 1640 supplemented with 10% heat-inactivated autologous serum). Monocytes were obtained by incubating PBMC suspensions in 10-cm plastic tissue culture dishes for 2 h at 37°C in a humidified 5% CO_2 incubator, as described previously [21], and the recovered cell population was routinely determined to be > 95% $CD14^+$ cells by flow cytometric analysis. Non-adherent cells, predominantly lymphocytes, were harvested by gently washing three times with warmed (37°C) culture medium. Highly purified T cells were obtained by passing the non-adherent cells through a nylon wool column once or twice to remove B lymphocytes and remaining monocytes [22]. Briefly, a 10 ml plastic syringe was packed with 0·5 g nylon wool (Wako Pure Chemical Industries Ltd, Osaka, Japan), drained with RPMI 1640 medium containing 5% autologous serum and equilibrated in a 37°C incubator for 60 min. To this column were added $1–3 \times 10^7$ cells in 2 ml medium. The column was incubated at 37°C for 60 min in a 5% CO_2 humidified atmosphere. The non-adherent cells were eluted with 14 ml of warmed medium (37°C) at a flow rate of one drop/s. The eluted cells were harvested and washed three times with culture medium. The resultant T cell preparations were found to be > 90% T cells ($CD3^+$), < 0·5% B cells ($CD19^+$) and < 0·5% monocytes ($CD14^+$) by analysis on a FACScan flow cytometer (Becton Dickinson, Mountain View, CA). The T cell preparations failed to proliferate to optimal doses of superantigens, indicating negligible contamination with APC.

Superantigens and monoclonal antibodies

SEB and staphylococcal toxic shock syndrome toxin-1 (TSST-1) were obtained from Sigma (St Louis, MO). Streptococcal pyogenic exotoxin A (SPEA) was obtained from Shionogi Pharmaceutical Co. (Osaka, Japan).

For flow cytometric analysis, monoclonal anti-human CD3 (Leu-4), CD4 (Leu-3a), CD8 (Leu-2a), CD14 (Leu-M3), CD19 (Leu-12), HLA-DR, LFA-1a (CD11a), CD18, ICAM-1 (CD54), and LFA-3 (CD58) were obtained from Becton Dickinson. Anti-human B7-1 (CD80) and anti-human B7-2 (CD86) were obtained from PharMingen (San Diego, CA). These antibodies were all conjugated with FITC or PE. For blocking studies, monoclonal anti-human HLA-DR (Nu-Ia) were obtained from Nichirei Co. (Tokyo, Japan). Anti-CD18 (MHM23), anti-CD11a (MHM24), anti-ICAM-1 (CD54), and anti-LFA-3 (CD58) were obtained from Dakopatts (Glostrup, Denmark). Anti-B7-2 was obtained from Ancell (Bayport, MN).

Preparation of supernatants of SEB-stimulated PBMC

The supernatants of PBMC stimulated with a bacterial superantigen SEB, were prepared. Briefly, PBMC suspensions were distributed into 24-well culture plates (Falcon, Oxnard, CA) at 1×10^6 cells/ml in a final volume of 1 ml, and SEB was added to each well at 1 μg/ml. After incubation for 48 h at 37°C, culture media were collected, centrifuged, and stored at −20°C until use. The supernatants were used in experiments at a final concentration of 20% (v/v).

Flow cytometric analysis of cell adhesion molecules on HT-29 cells and monocytes

To determine the expression and up-regulation of HLA-DR and costimulatory molecules on the surface of HT-29 cells, the cells ($1–2 \times 10^5$/ml per well) were cultured in a 24-well tissue culture plate (Falcon) for 48 h with or without the prepared supernatants of SEB-stimulated PBMC in a final concentration of 20%. In some experiments, recombinant human IFN-γ (Genzyme, Cambridge, MA) and tumour necrosis factor-alpha (TNF-α; R&D Systems, Minneapolis, MN) were used as stimulators at a concentration of

500 U/ml and 50 ng/ml, respectively. The cells were detached with 0·1% trypsin and 0·5 mM EDTA, and washed three times with culture medium. For immunofluorescence staining, HT-29 cells with or without stimulation and monocytes (1×10^6 cells) were washed three times with PBS containing 0·2% bovine serum albumin (BSA) and 0·1% NaN_3 for 3 min at 4°C, then directly incubated with FITC- or PE-conjugated MoAbs. After incubation on ice for 30 min, samples were washed three times again with the same solution, and then fixed in PBS containing 1% paraformaldehyde. The expression of cell adhesion molecules on HT-29 cells and monocytes was analysed using a FACScan flow cytometer (Becton Dickinson). Unstained cells and cells incubated with isotype-matched irrelevant FITC- or PE-conjugated control MoAbs were included in each experiment as controls.

Paraformaldehyde fixation of APC

HT-29 cells with or without stimulation and monocytes were used as APC. To assess their APC function for superantigen-induced T cell proliferation, cells were treated with freshly prepared 1% (w/v) paraformaldehyde solution in PBS for 15 min at room temperature, washed three times with PBS and used as fixed APC [23].

Lymphocyte proliferation assays

The fixed APC, monocytes or HT-29 cells (5×10^4/well) were co-cultured with the purified T cells (1×10^5/well) in triplicate in 96-well round-bottomed microwell plates (Falcon) in the presence or absence of bacterial superantigens SEB, TSST-1, and SPEA (1–100 ng/ml) in 0·2 ml of medium for 4 days at 37°C in an atmosphere of 5% CO_2, and 0·5 μCi of ^{3}H-thymidine per well was added in the last 18 h. The cells were harvested onto glassfibre filter mats using a PHD cell harvester (Cambridge Technologies, Cambridge, MA), and the incorporation of ^{3}H-thymidine was determined by standard liquid scintillation techniques. Results were expressed in ct/min as the mean ct/min ± s.d. of triplicate culture. T cells plus superantigens without APC and APC plus T cells without superantigens were included in each experiment as controls. Control wells containing HT-29 cells and T cells showed no significant mixed lymphocyte reaction (MLR) at 4 days.

Inhibition of T cell proliferation by blocking MoAbs

A panel of MoAbs against HLA-DR, ICAM-1, B7-2, LFA-3, and control mouse IgG, were used for blocking experiments. The fixed monocytes or HT-29 cells (5×10^4/well) were preincubated with the indicated MoAbs (10 μg/ml) for 30 min at 37°C before adding superantigen SEB and T cells (1×10^5/well). All cultures were performed in triplicate in 96-well round-bottomed microtitre plates (Falcon) for 4 days, and T cell proliferation was assayed as described above.

ELISA for IL-8

To determine IL-8 production, HT-29 cells were seeded at $1–2\times10^5$ cells/ml into 24-well tissue culture plates (1 ml/well) and incubated with or without the supernatants of SEB-stimulated PBMC for 48 h, after which the medium was changed for fresh medium, and then incubated with or without superantigen SEB (1–100 ng/ml) or human recombinant TNF-α (1–100 ng/ml) for 24 h. In some experiments, purified T cells (1×10^6 cells/well) were co-cultured. After incubation for 24 h, the media were collected, cleared by centrifugation and passage through 0·22 μm sterile filters and kept at −80°C until evaluation by ELISA (R&D Systems).

Statistical analysis

Results were expressed as mean ± s.d. The statistical significance of differences between the means was performed using Student's *t*-test. $P < 0·05$ was considered statistically significant.

RESULTS

Expression and up-regulation of costimulatory molecules and HLA-DR on HT-29 cells by the supernatants of SEB-stimulated PBMC

A single representative from seven different experiments is shown in Fig. 1. In peripheral blood monocytes there was significant expression of HLA-DR, ICAM-1, LFA-1 (CD11a/CD18), LFA-3 and B7-2 (CD86), but no constitutive expression of B7-1 (CD80) molecules (data not shown). In HT-29 cells without stimulation, there was constitutive expression of ICAM-1 and LFA-3 molecules at a low level (≈ 25% and ≈ 12%, respectively), but no detectable basal level of HLA-DR, LFA-1 (CD11a/CD18), B7-1 (CD80), or B7-2 (CD86) molecules was observed. After HT-29 cells were stimulated with the supernatants of SEB-stimulated PBMC for 48 h, there was significantly increased expression of HLA-DR and ICAM-1 molecules on HT-29 cells. Most stimulated HT-29 cells were HLA-DR- and ICAM-1-positive (both > 90%). However, this stimulation had no effect on expression of LFA-1, B7-1, B7-2 and LFA-3 molecules.

In the next experiment, the regulation of these molecules on HT-29 cells by IFN-γ and TNF-α was investigated. As shown in Table 1, IFN-γ significantly induced the expression of HLA-DR and ICAM-1, reaching a peak level at 48 h and 24 h, respectively. IFN-γ seemed to have a similar ability to the supernatants of SEB-stimulated PBMC in up-regulating HLA-DR and ICAM-1 expression. TNF-α also significantly induced the expression of ICAM-1 at 24 h; however, it seemed to have little effect on the expression of HLA-DR. No synergistic effect of IFN-γ and TNF-α on induction of HLA-DR and ICAM-1 was observed (data not shown). Both IFN-γ and TNF-α stimulation had no effect on the expression of LFA-1, LFA-3, B7-1 and B7-2 molecules (data not shown). In all subsequent experiments, the supernatants of SEB-stimulated PBMC were used as a stimulator for HT-29 cells to up-regulate HLA-DR and ICAM-1 expression.

Stimulated HT-29 cells function as APC for bacterial superantigens

As shown in Fig. 2, peripheral blood monocytes, as a positive control, could significantly present all tested bacterial superantigens SEB, TSST-1 and SPEA, to purified T cells and caused significant T cell proliferation. In the presence of SEB, TSST-1 and SPEA (1–100 ng/ml), HT-29 cells without stimulation did not cause T cell proliferation. Stimulated HT-29 cells, however, caused significant T cell proliferation in a superantigen dose-dependent manner ($P < 0·05$). In this experimental system, control wells containing stimulators (monocytes or HT-29 cells) or responders (T cells) showed no significant ^{3}H-thymidine incorporation, even though optimal concentration of superantigens was added.

Inhibition of T cell proliferation by MoAbs against HLA-DR and costimulatory molecules.

To explore the molecular basis of HT-29 cells as APC for superantigens and compare them with peripheral monocytes, SEB was selected to use for a representative superantigen, and a panel of MoAbs was used to investigate their effects on SEB-induced T cell

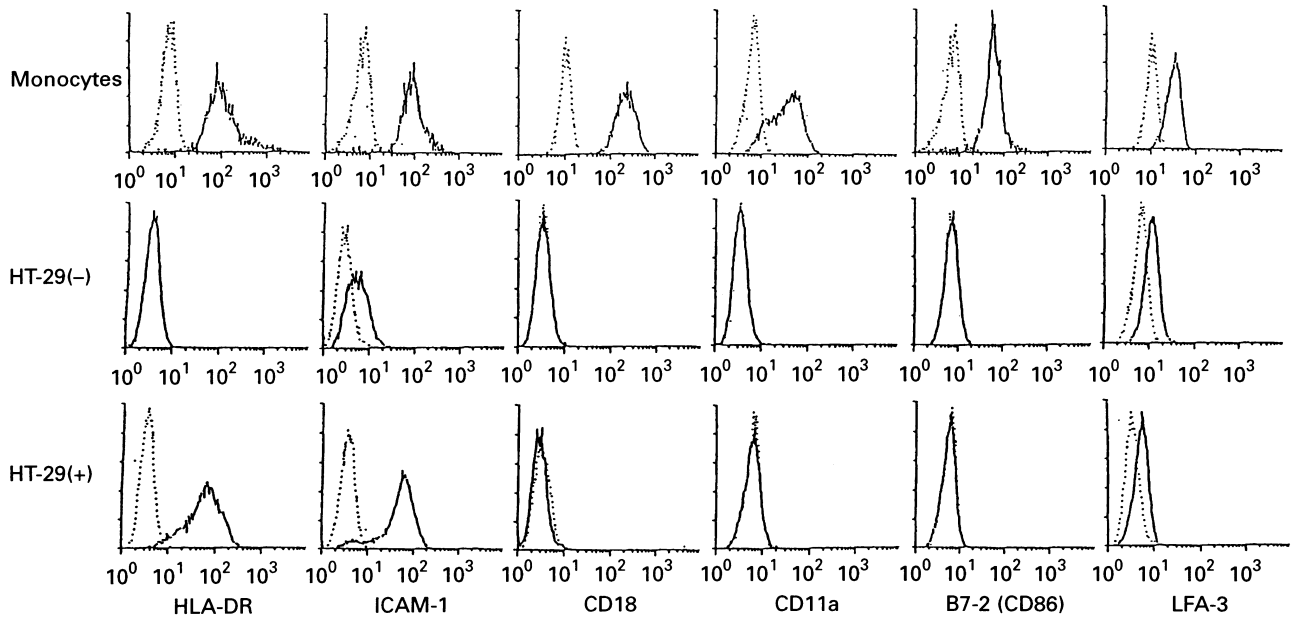

Fig. 1. Flow cytometric analysis for the expression of costimulatory molecules and HLA-DR on a human colonic epithelial cell line HT-29 and peripheral blood monocytes. HT-29 cells, without stimulation or stimulated by the supernatants of staphylococcal enterotoxin B (SEB)-stimulated peripheral blood mononuclear cells (PBMC) for 48 h at 37°C, were stained with a panel of PE- or FITC-conjugated MoAbs against HLA-DR, intercellular adhesion molecule-1 (ICAM-1) (CD54), CD11a, CD18, B7-2 (CD86) and LFA-3 (CD58). Cells were analysed by a FACScan flow cytometer. HT-29(–), No stimulation; HT-29(+), stimulated with the supernatants of SEB-stimulated PBMC; ·····, control MoAbs.

proliferation. Based on the results of flow cytometric analysis, the relative contributions of HLA-DR, ICAM-1, B7-2 and LFA-3 were specifically explored. As shown in Figs 3 and 4, MoAbs against HLA-DR, B7-2 and LFA-3 showed a significant inhibition of T cell proliferation ($P < 0{\cdot}05$) when monocytes were used as APC, but the MoAb against ICAM-1 had no effect on this response. On the other hand, when stimulated HT-29 cells were used as APC, MoAbs against HLA-DR and ICAM-1 significantly inhibited SEB-induced T cell proliferation at all tested concentrations of SEB (1–100 ng/ml), and the inhibition of T cell proliferation by anti-HLA-DR and anti-ICAM-1 was > 85% and 60–75%, respectively. When these two MoAbs were added together, no significant synergistic inhibition was observed. In contrast to monocytes, MoAbs against B7-2 and LFA-3 had no effect on SEB-induced T cell proliferation. Control IgG had no effect on this response.

IL-8 production of HT-29 cells stimulated with SEB

TNF-α significantly induced HT-29 cells to produce high levels of IL-8 in a dose-dependent manner whether HT-29 cells were stimulated with the supernatants of SEB-stimulated PBMC or not (Fig. 5), and IL-8 production by the stimulated HT-29 cells was higher than that of non-stimulated HT-29 cells ($P < 0{\cdot}05$). On the other hand, SEB could not directly induce the production of IL-8 by HT-29 cells, irrespective of whether HT-29 cells were stimulated or not (Fig. 6). However, when T cells were added, SEB could induce stimulated HT-29 cells, but not non-stimulated HT-29 cells, to produce IL-8 significantly ($P < 0{\cdot}05$).

Table 1. Up-regulation of HLA-DR and intercellular adhesion molecule-1 (ICAM-1) on HT-29 cells by IFN-γ and tumour necrosis factor-alpha (TNF-α)

	HLA-DR (%)			ICAM-1 (%)		
	0 h	24 h	48 h	0 h	24 h	48 h
IFN-γ (500 U/ml)	0·9	17·8	81·0	23·4	85·8	86·4
TNF-α (50 ng/ml)	0·9	1·1	12·0	23·4	84·6	81·3

HT-29 cells were cultured with IFN-γ and TNF-α for indicated time, harvested and stained with FITC-conjugated HLA-DR MoAb and PE-conjugated ICAM-1 MoAb, then processed to flow cytometric analysis. Data are expressed as percent of positive cells.

DISCUSSION

In the present study, the constitutive expression of HLA-DR molecules and a panel of costimulatory molecules B7-1, B7-2, ICAM-1, LFA-1 and LFA-3 on an intestinal epithelial cell line HT-29, and their up-regulation by the supernatants of a bacterial superantigen SEB-stimulated PBMC were investigated. The results showed that ICAM-1 and LFA-3 were constitutively expressed at a low level, and no constitutive expression of HLA-DR and LFA-1 was observed on the surface of HT-29 cells. These results are consistent with previously published studies by different methods [11,24–26]. Proinflammatory cytokines IFN-γ, TNF-α and IL-1 can increase expression of HLA-DR and costimulatory molecules [24–26]. Superantigens can activate a great number of T cells to release a variety of cytokines, including these proinflammatory cytokines [1,2]. Most HT-29 cells were found to express HLA-DR and ICAM-1 molecules (both > 90%) when stimulated by the supernatants of SEB-stimulated PBMC, indicating that T cell activation by superantigen can regulate the function of IEC. IFN-γ significantly increased expression of HLA-DR and ICAM-1 molecules, which increased expression sufficiently to reach levels similar to that by the supernatants of SEB-stimulated PBMC, suggesting that up-regulation by supernatants of SEB-stimulated

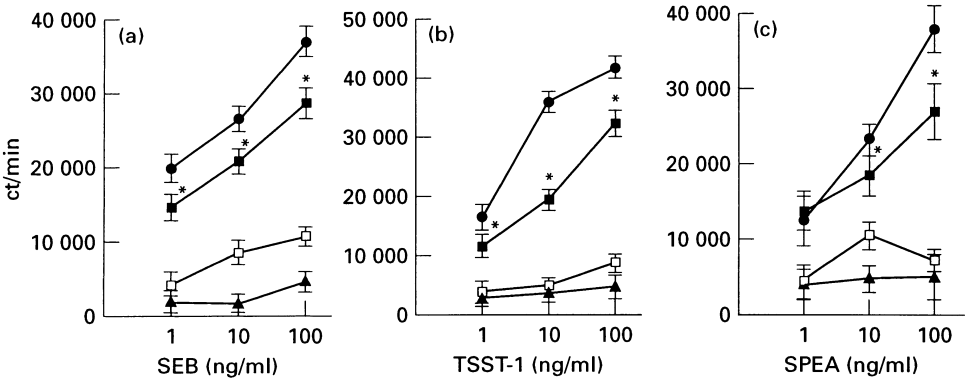

Fig. 2. Peripheral blood T lymphocyte proliferation in response to bacterial superantigens staphylococcal enterotoxin B (SEB) (a), toxic shock syndrome toxin-1 (TSST-1) (b), and streptococcal pyogenic exotoxin A (SPEA) (c). T cells (1×10^5) were co-cultured with 5 x10^4 fixed peripheral blood monocytes or HT-29 cells for 4 days in the presence of bacterial superantigens at the dose indicated. ^{3}H-thymidine was added for the last 18 h, and ^{3}H-thymidine incorporation (ct/min) was measured as described in Materials and Methods. HT(−), No stimulation; HT(+), stimulated with the supernatants of SEB-stimulated peripheral blood mononuclear cells (PBMC). *P < 0·05 *versus* T + HT(−). ●, T + monocytes; ■, T + HT(+); □, T + HT(−); ▲, T alone.

PBMC may predominantly result from IFN-γ, and it is possible that superantigens induce local T lymphocyte activation and regulate the function of IEC *in vivo*. In agreement with one report by Kvale *et al.* [24], we found that both the supernatants of SEB-stimulated PBMC and proinflammatory cytokines (IFN-γ and TNF-α) had no effect on LFA-3 expression on HT-29 cells, although a low level of LFA-3 was constitutively expressed.

B7-1 and B7-2 are major costimulatory molecules expressed on APC, which recognize CD28 on T cells, and provide important costimulation for T cell activation and proliferation [27]. Sanderson *et al.* [28] reported that no mRNA expression of B7-1 in mouse enterocytes was observed. A recent immunohistochemical study also did not find B7-1 expression on normal or inflamed human colonic epithelium [26]. Our results showed no constitutive expression of either B7-1 or B7-2 on HT-29 cells using flow cytometric analysis, and the supernatants of SEB-stimulated PBMC or proinflammatory cytokines (IFN-γ and TNF-α) could not induce their expression. These findings indicate that IEC lack the expression of both B7-1 and B7-2 molecules.

Several kinds of non-lymphoid cells, such as epidermal keratinocytes and vascular endothelial cells, have been reported to be able to function as APC for bacterial superantigens [16,29]. These cells could be induced to express HLA-DR by treatment with IFN-γ, and obtained the ability to present superantigens. In keeping with these reports, our study showed that stimulated HT-29 cells, which expressed HLA-DR and ICAM-1 molecules,

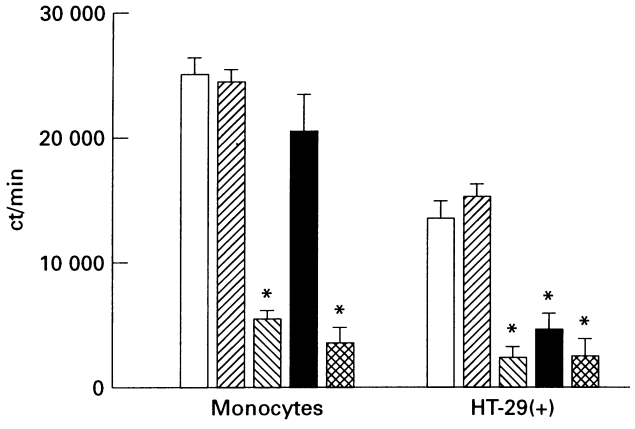

Fig. 3. The effects of anti-HLA-DR and anti-intercellular adhesion molecule-1 (ICAM-1) MoAbs on T cell proliferation to staphylococcal enterotoxin B (SEB) presented by peripheral blood monocytes and stimulated HT-29 cells. Monocytes (5×10^4) or stimulated HT-29 cells were preincubated with anti-HLA-DR or anti-ICAM-1 (CD54) MoAb (10 μg/ml) for 30 min, then co-cultured with purified peripheral blood T cells (1×10^5) for 4 days in the presence of SEB (100 ng/ml), and ^{3}H-thymidine incorporation (ct/min) was measured. Representative of three separate experiments. HT-29(+), Stimulated with the supernatants of SEB-stimulated peripheral blood mononuclear cells (PBMC). *P < 0·05 *versus* control MoAb. □, Antibody(−); ▨, control IgG; ▧, anti-HLA-DR; ■, anti-ICAM-1; ▩, anti-HLA-DR + anti-ICAM-1.

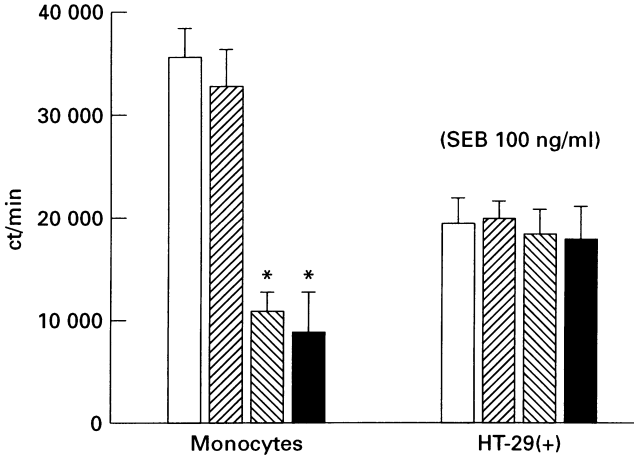

Fig. 4. The effects of anti-B7-2 and anti-LFA-3 MoAbs on T cell proliferation to staphylococcal enterotoxin B (SEB) presented by peripheral blood monocytes and stimulated HT-29 cells. Monocytes or stimulated HT-29 cells (5×10^4) were preincubated with anti-B7-2 (CD86) or anti-LFA-3 (CD58) MoAb (10 μg/ml) for 30 min, then co-cultured with purified peripheral blood T cells (1×10^5) for 4 days in the presence of SEB (100 ng/ml), and ^{3}H-thymidine incorporation (ct/min) was measured. Representative of three separate experiments. HT-29(+), Stimulated with the supernatants of SEB-stimulated peripheral blood mononuclear cells (PBMC). *P < 0·05 *versus* control MoAb. □, Antibody(−); ▨, control IgG; ▧, anti-B7-2 (CD86); ■, anti-LFA-3.

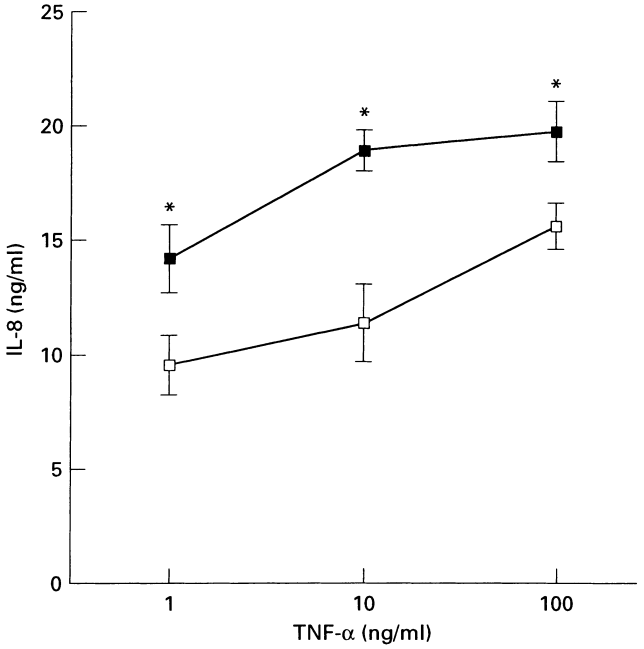

Fig. 5. Tumour necrosis factor-alpha **(**TNF-α)-induced IL-8 production by HT-29 cells. HT-29 cells, with or without stimulation by the supernatants of staphylococcal enterotoxin B (SEB)-stimulated peripheral blood mononuclear cells (PBMC), were cultured for 24 h in the presence of TNF-α, then the amount of IL-8 in supernatants was determined by ELISA as described in Materials and Methods. HT-29(−) (□), No stimulation; HT-29(+) (■), stimulated with the supernatants of SEB-stimulated PBMC. *$P < 0{\cdot}05$ *versus* HT-29(−).

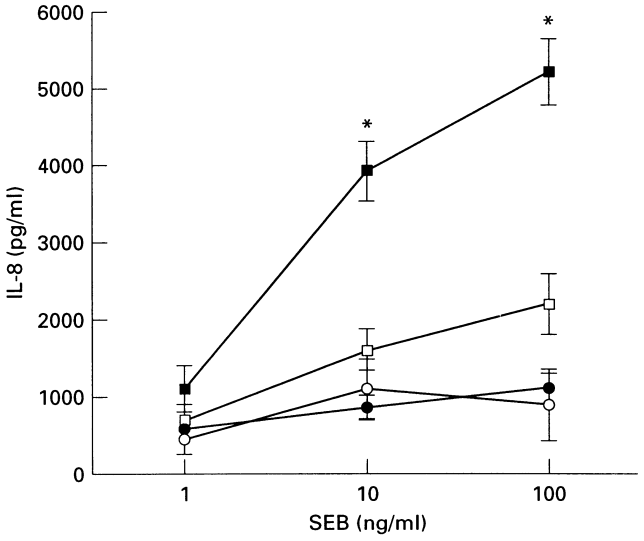

Fig. 6. Staphylococcal enterotoxin B (SEB)-induced IL-8 production when HT-29 cells were used as antigen-presenting cells. HT-29 cells, with or without stimulation by the supernatants of SEB-stimulated peripheral blood mononuclear cells (PBMC), were cultured with SEB (1–100 ng/ml) for 24 h in the absence or presence of purified peripheral blood T cells, then the amount of IL-8 in supernatants was determined by ELISA. HT(−), No stimulation; HT(+), stimulated with the supernatants of SEB-stimulated PBMC. *$P < 0{\cdot}05$ *versus* HT(−). ■, HT(+) + T; □, HT(−) + T; ●, HT(+); ○, HT(−).

could serve as APC for SEB, TSST-1 and SPEA, and caused significant T cell proliferation. In the antibody blocking experiments, anti-HLA-DR MoAb significantly inhibited SEB-induced T cell proliferation when stimulated HT-29 cells were used as APC (inhibition by > 85%), indicating that HLA-DR are essential molecules in superantigen-induced T cell proliferation. A recent report by Aisenberg *et al.* [30] showed that about 50% of freshly isolated IEC preparations from normal individuals (seven of 14 individual IEC preparations) was capable of presenting superantigens SEB, SEE and TSST-1 to peripheral blood T cells, suggesting a possibility of IEC to function as APC *in vivo* for bacterial superantigens.

It has been previously shown that ICAM-1-transfected cells enhance T cell proliferation [31–33]. In the present study, ICAM-1 was constitutively expressed on HT-29 cells at a low level, and expression was markedly induced after stimulation (> 90% positive). The stimulated HT-29 cells could serve as APC to present bacterial superantigens, and MoAb against ICAM-1 could significantly inhibit T cell proliferation (inhibition by 60–75%). This result suggests that ICAM-1 on the surface of HT-29 cells may play an important role in superantigen-induced T cell proliferation through providing costimulation. Consistent with the results, several studies using endothelial cells and keratinocytes also showed that anti-ICAM-1 MoAb significantly inhibited superantigen-induced T cell proliferation [16,29]. On the other hand, we did not find expression of B7-1 and B7-2 on HT-29 cells, and antibody against B7-2 had no effect on SEB-induced T cell proliferation. Although there was a low level of LFA-3 expression on HT-29 cells, the antibody against LFA-3 had no effect on SEB-induced T cell proliferation. These results suggest that B7-2- and LFA-3-mediated costimulation are not involved in T cell responses initiated by IEC, which was in contrast to superantigen-induced T cell responses when monocytes were used as APC.

The interaction between IEC and T lymphocytes, and T lymphocyte activation by IEC, play important roles in mucosal immunoregulation. IEC are exposed *in vivo* to a wide variety of bacteria and bacterial products, including bacterial superantigens that pass through the intestinal lumen. Invasion of the intestinal mucosa by pathogenic bacteria leads to a markedly acute mucosal inflammatory response. IEC can be induced to produce chemokines such as IL-8 and monocyte chemoattractant protein-1 (MCP-1) [12–14], which can attract an infiltration of neutrophils and monocytes/macrophages. A report showed that the synoviocytes, a kind of non-professional APC, can produce IL-8 by direct superantigen binding to HLA-DR molecules on these cells induced by IFN-γ [34]. In contrast to this report, the present study did not find that a superantigen, SEB, can directly stimulate HT-29 cells to produce IL-8, whether HT-29 cells expressed HLA-DR or not. However, we found that stimulated HT-29 cells with the expression of HLA-DR and ICAM-1 could present superantigen to T cells, and in this condition HT-29 cells could produce IL-8 significantly. This may be attributed to the secondary stimulation of cytokines from activated T cells by superantigen. On the other hand, a proinflammatory cytokine, TNF-α, produced by activated immune cells can significantly stimulate HT-29 cells to produce IL-8. Furthermore, stimulated HT-29 cells produce much more IL-8 than non-stimulated HT-29 cells in response to TNF-α. We speculate that this may be correlated with increased TNF-α receptor expression in stimulated HT-29 cells. Recent reports by Aisenberg *et al.* showed that T cells of human intestinal mucosa, including CD and ulcerative colitis patients, proliferated in

response to superantigens [30], and superantigens can also induce intestinal mucosal lymphocytes to produce proinflammatory cytokines [35]. These findings suggest that superantigens may mediate mucosal inflammation *in vivo*, and IEC may directly participate in inflammatory responses and modulate T cell function in bacterial superantigen-related acute infection and chronic intestinal diseases, including IBD.

In conclusion, the present study demonstrates that the products of T cell activation by superantigen could significantly increase HLA-DR and ICAM-1 expression on an IEC cell line, HT-29, which may predominantly result from IFN-γ. The stimulated HT-29 cells can function as accessory cells for T cell proliferation induced by superantigens. HLA-DR molecules are essential in HT-29 cell-mediated T cell proliferative response; in addition, ICAM-1 molecules also play an important role by providing costimulation. These *in vitro* results provide new insights into how IEC can actively participate in the intestinal immune response *in vivo*, and may have implications for our understanding of the pathogenesis of superantigen-related intestinal infectious diseases and chronic IBD.

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References

1 Marrack P, Kappler J. The staphylococcal enterotoxins and their relatives. Science 1990; **248**:705–10.
2 Schlievert PM. Role of superantigens in human disease. J Infect Dis 1993; **167**:997–1002.
3 Conrad B, Weidmann E, Trucco G *et al.* Evidence for superantigen involvement in insulin-dependent diabetes mellitus aetiology. Nature 1994; **371**:351–5.
4 Posnett DN, Schmelkin I, Burton DA, August A, McGrath H, Mayer LF. T cell antigen receptor usage: increases in Vβ8$^+$ T cells in Crohn's disease. J Clin Invest 1990; **85**:1770–6.
5 Baca-Estrada ME, Wong DKH, Croitoru K. Cytotoxic activity of Vβ8$^+$ T cells in Crohn's disease: the role of bacterial superantigens. Clin Exp Immunol 1995; **99**:398–403.
6 Kay RA. The potential role of superantigens in inflammatory bowel disease. Clin Exp Immunol 1995; **100**:4–6.
7 Bland PW, Warren LG. Antigen presentation by epithelial cells of the rat small intestine. I. Kinetics, antigen specificity and blocking by anti-Ia antisera. Immunology 1986; **58**:1–7.
8 Mayer L, Shilen R. Evidence for function of Ia molecules on gut epithelium in man. J Exp Med 1987; **166**:1471–83.
9 Selby WS, Janossy G, Mason DY, Jewell DP. Expression of HLA-DR antigens by colonic epithelium in inflammatory bowel disease. Clin Exp Immunol 1983; **53**:614–8.
10 Mayer L, Eisenhardt D, Salomon P, Bauer W, Plous R, Piccinini L. Expression of class II molecules on intestinal epithelial cells in humans: differences between normal and inflammatory bowel disease. Gastroenterology 1991; **100**:3–12.
11 Sollid LM, Gaudernack G, Markussen G, Kvale D, Brandtzaeg P, Thorsby E. Induction of various HLA class II molecules in a human colonic adenocarcinoma cell line. Scand J Immunol 1987; **25**:175–80.
12 Eckmann L, Jung HC, Schürer-Maly C, Panja A, Morzycka-Wroblewska E, Kagnoff MF. Differential cytokine expression by human intestinal epithelial cell lines: regulated expression of interleukin 8. Gastroenterology 1993; **105**:1689–97.
13 Reinecker HC, Loh EY, Ringler DJ, Methta A, Rombeau JL, MacDermott RP. Monocyte-chemoattractant protein 1 gene expression in intestinal epithelial cells and inflammatory bowel disease mucosa. Gastroenterolgy 1995; **108**:40–50.
14 Jung HC, Eckmann L, Yang SK *et al.* A distinct array of proinflammatory cytokines is expressed in human colon epithelial cells in response to bacterial invasion. J Clin Invest 1995; **95**:55–65.
15 Parra E, Wingren AG, Hedlund G *et al.* Costimulation of human CD4$^+$ T lymphocytes with B7 and lymphocyte function-associated antigen-3 results in distinct cell activation profiles. J Immunol 1994; **153**:2479–87.
16 Nickoloff BJ, Mitra RS, Green J *et al.* Accessory cell function of keratinocytes for superantigens. Dependence on lymphocyte function-associated antigen-1/intercellular adhesion molecule-1 interaction. J Immunol 1993; **150**:2148–59.
17 Van Seventer GA, Newman W, Shimizu Y *et al.* Analysis of T cell stimulation by superantigen plus major histocompatibility complex class II molecules or by CD3 monoclonal antibody: costimulation by purified adhesion ligand VACM-1, ICAM-1, but not ELAM-1. J Exp Med 1991; **174**:901–13.
18 Kaiserlian D, Rigal D, Abello J, Revillard JP. Expression, function and regulation of the intercellular adhesion molecule-1 (ICAM-1) on human intestinal epithelial cell lines. Eur J Immunol 1991; **21**:2415–21.
19 Kelly CP, O'Keane JC, Opellana J *et al.* Human colon cancer cells express ICAM-1 *in vivo* and support LFA-1-dependent lymphocyte adhesion *in vitro*. Am J Physiol 1992; **263**:G864–70.
20 Chantret I, Barbat A, Dussualx E, Brattain MG, Zweibaum A. Epithelial polarity, villin expression, and enterocytic differentiation of cultured human colon carcinoma cell lines: survey of 20 cell lines. Cancer Res 1988; **48**:1936–42.
21 Liu ZX, Noguchi M, Hiwatashi N, Toyota T. Monocyte aggregation and multinucleated giant-cell formation *in vitro* in Crohn's disease: the effect of cell adhesion molecules. Scand J Gastroenterol 1996; **31**:706–10.
22 Abo T, Sugawara S, Amenomori A *et al.* Selective phagocytosis of Gram-positive bacteria and interleukin 1-like factor production by a subpopulation of large granular lymphocytes. J Immunol 1986; **136**:3189–97.
23 Moreno J, Lipsky PE. Differential ability of fixed antigen-presenting cells to stimulate normal antigen-reactive and alloreactive T 4 lymphocytes. J Immunol 1986; **136**:3579–87.
24 Kvale D, Krajci P, Brandtzaeg P. Expression and regulation of adhesion molecules ICAM-1 (CD54) and LFA-3 (CD58) in human intestinal epithelial cell lines. Scand J Immunol 1992; **36**:669–76.
25 Dippold W, Wittig B, Schwaeble W, Mayet W, Meyer zum Büschenfelde KH. Expression of intercellular adhesion molecule 1 (ICAM-1, CD54) in colonic epithelial cells. Gut 1993; **34**:1593–7.
26 Bloom S, Simmons D, Jewell DP. Adhesion molecules intercellular adhesion molecule-1 (ICAM-1), ICAM-3 and B7 are not expressed by epithelium in normal or inflamed colon. Clin Exp Immunol 1995; **101**:157–63.
27 June CH, Bluestone JA, Nadler LM, Tompson CB. The B7 and CD28 receptor families. Immunol Today 1994; **15**:321–31.
28 Sanderson IR, Ouellette AJ, Carter EA, Walker WA, Harmatz PR. Differential regulation of B7 mRNA in enterocytes and lymphoid cells. Immunology 1993; **79**:434–8.
29 Krakauer T. Costimulatory receptor for the superantigen staphylococcal enterotoxin B on human vascular endothelial cells and T cells. J Leukocyte Biol 1994; **56**:458–63.
30 Aisenberg J, Ebert EC, Mayer L. T-cell activation in human intestinal mucosa: the role of superantigens. Gastroenterology 1993; **105**:1421–30.
31 Mondino A, Jenkins MK. Surface proteins involved in T cell co-stimulation. J leukocyte Biol 1994; **55**:805–15.
32 Dubey C, Croft M, Swain SL. Costimulatory requirements of naive CD4$^+$ T cells: ICAM-1 or B7-1 can costimulate naive CD4 T cell activation but are required for optimum response. J Immunol 1995; **155**:45–57.

33 Hagerty DT. Intercellular adhesion molecule-1 is necessary but not sufficient to activate $CD4^+$ T cells: discovery of a novel costimulator on kidney tubule cells. J Immunol 1996; **156**:3652–9.

34 Mourad W, Mehindate K, Schall TJ, McColl SR. Engagement of major histocompatibility complex class II molecules by superantigen induces inflammatory cytokine gene expression in human rheumatoid fibroblast-like synoviocytes. J Exp Med 1992; **175**:613–6.

35 Sperber K, Silverstein L, Brusco C, Yoon C, Mullin GE, Mayer L. Cytokine secretion induced by superantigens in peripheral blood mononuclear cells, lamina propria lymphocytes, and intraepithelial lymphocytes. Clin Diagn Lab Immunol 1995; **2**:473–7.