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Synergistic interactions of CD4+ and CD8+ T cell subsets with human vascular endothelial cells in primary proliferative allogeneic responses

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Abstract

The ability of subcultured human vascular endothelial cells (EC) to provide immune accessory functions for proliferative responses of highly purified allogeneic CD4+ and CD8+ T cells has been examined. CD4+ T cells proliferated in response to IFN-γ-pretreated EC which expressed class II molecules, but not to untreated EC. CD8+ T cells proliferated to MHC class I molecules expressed on both untreated and IFN-γ-treated EC. Combined populations of CD4+ and CD8+ T cells showed synergistic, rather than additive, responses to both untreated and IFN-γ-treated EC. Furthermore, CD8+ T cells were able to induce MHC class II expression on endothelial cells and this induction could be inhibited by an anti-IFN-γ mAb. The synergistic response obtained by co-culturing CD4+ and CD8+ T cells with vascular EC was completely inhibited by the same anti-IFN-γ mAb. These studies suggest that CD4+ and CD8+ T cells recognise and proliferate to allogeneic MHC molecules expressed by EC. CD4+ and CD8+ responses are synergistic under the conditions tested and this synergism appears to be due to induction of MHC class II antigens on EC by IFN-γ secreted from CD8+ T cells.

Introduction

Endothelial cells (EC) have a diverse range of metabolic functions. At sites of inflammation, the EC that line postcapillary venules are able to undergo a number of structural and functional changes. They become more adhesive towards leucocytes and show altered surface expression of proteins that enable leucocyte interactions. The EC expression of MHC class I and II molecules which promote cellular immune responses are also increased. These changes have been collectively referred to as endothelial activation and are considered to be caused by locally acting cytokines (1).

In 1975, Hirschberg and colleagues suggested a role for EC in cell-mediated immune responses following studies that examined the ability of EC to stimulate allogeneic lymphocytes *in vitro* (2). The demonstration that IFN-γ can upregulate the EC expression of MHC class I antigens and induce EC expression of MHC class II antigens *de novo*, suggested that IFN-γ might play a critical role in these responses (3). Furthermore, the fact that IFN-γ-treated EC were able to stimulate allogeneic CD4+ T cells while IFN-γ-treated dermal fibroblasts that expressed equivalent levels of MHC class II molecules (HLA-DR) were not, suggested that EC were able to provide additional signals to support alloproliferation (4). Endothelial cells appear to provide part of the necessary additional signals via LFA-3, which interacts with CD2 on the surface of CD4+ T cells; this interaction appears to stimulate CD4+ T cells to secrete augmented levels of the cytokine IL-2 (an autocrine growth factor) (5,6). Interactions between LFA-3 and CD2, as well as interactions between the TCR and MHC class II molecules, are critical to primary allogeneic proliferative responses of CD4+ T cells to IFN-γ-treated cultured EC, since blocking the LFA-3:CD2 binding or the TCR:MHC class II binding by mAb inhibits proliferation (6). Other molecules that are permissive for these primary allogeneic proliferative responses include T cell LFA-1 and CD44, which is expressed by both T cells and EC. In this paper the primary allogeneic proliferative responses of CD4+ and CD8+ T cells to untreated and to IFN-γ-treated endothelium are examined to define how lymphocyte subsets may interact with endothelium in the peripheral vasculature at inflammatory sites.

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Methods

Isolation of EC

EC were isolated from human umbilical cord veins under sterile conditions using previously described methods (7,8). To exclude the presence of contaminating leucocytes EC were subcultured and maintained in serial culture using a combination of heparin and endothelial cell growth factor (9). Endothelial cell growth factor was prepared according to methods described by Maciag and Weinstein (10). Endothelial cells were used at a final passage of between three and five and plated at a confluent density (2×10^4 cells/0.2 ml tissue culture well; 1×10^5 cells/2.0 ml well). Tissue culture plates (Becton-Dickinson, Plymouth, UK) were coated using 0.1% gelatin.

The purity of the EC populations for MHC class II expressing cells and for cells expressing the common leucocyte antigen, CD45, was determined using indirect immunofluorescence techniques and by FACS analysis, and no class II or CD45 expressing cells were detected. The EC were also shown to be free of contaminating MHC class II expressing cells using a reverse transcriptase polymerase chain reaction to amplify HLA-DR RNA as previously described (6). As a functional check for EC purity we have confirmed that L-leucine methyl ester, a monocyte toxin, inhibits the ability of an adherent peripheral blood mononuclear cell population to stimulate a mixed lymphocyte response, but this toxin does not inhibit the ability of EC to stimulate a primary allogeneic proliferative response (6).

Isolation of CD4⁺ and CD8⁺ T cell subsets

Density gradient centrifugation using Ficoll-Hypaque (11) was used to isolate human peripheral blood mononuclear cells from heparinized blood taken from adult volunteers. B cells and monocytes were harvested by adherence to fibronectin coated bacteriological grade Petri dishes, and non-adherent cells (PBL) were gently removed from the fibronectin coated plates after a 45 min incubation period at 37°C. CD4⁺ or CD8⁺ T cells were negatively selected by labelling PBL with the following mAbs: OKM1 (IgG2b, anti-CD11b, a gift from P. Beverley, University College and Middlesex School of Medicine, London, UK), 3G8 (IgG1, anti-CD16, a gift from J. S. Pober, Yale University, CT), CD14 and CD19 (both IgG1, Serotec Ltd, Kidlington, Oxford, UK), L243 (anti-MHC class II, a gift from D. Altmann, Transplantation Biology, CRC, Harrow, UK) and either OKT8 (IgG2a, anti-CD8 isolated from ascites produced using OKT8 cells obtained from Porton Products, Porton Down, Salisbury, UK) or an anti-CD4 mAb (IgG1, Becton-Dickinson Monoclonal Antibody Center, Mountain View, CA, USA). The PBL were then incubated at 4°C for 45 min with the described antibodies each used at 10 μg/ml. At the end of this incubation period the cells were extensively washed and highly purified populations of CD4⁺ or CD8⁺ T cells were obtained by negative depletion with three successive incubations at 4°C with sheep anti-mouse Ig-coated magnetic beads [Dynabeads M-450, Dynal (UK) Ltd, Merseyside, UK] used at a 1:1 cell to bead ratio (12,13). Following these three incubation steps, the cell suspension was counted and washed in RPMI before being resuspended at a density of 3×10^6 cells/ml in RPMI supplemented with 10% heat inactivated FCS, 2.8 mM glutamine, penicillin and streptomycin (each at 100 units/ml) and 1.4×10^{-4} μM 2-mercaptoethanol. Absence of contaminating monocytes and dendritic cells was shown by the inability of our T cell preparations to respond to the bacterial super-antigen staphylococcal enterotoxin B (which binds MHC class II molecules on accessory cells to form a ligand capable of stimulating strong T cell proliferation) at concentrations between 0.01 and 1.0 ng/ml.

Co-culture of T cells with EC

EC monolayers were pretreated with 500 – 1000 IU/ml IFN-γ for 72 h to induce MHC class II antigens (3). Cells used in proliferative studies were pretreated for 1 h with 10 μg/ml mitomycin C (Sigma Chemical Company, Poole, Dorset, UK) in HBSS to inhibit further EC growth. T cells were added at a plating density of 3×10^5 cells/well for 96-well plates and at 3×10^6 cells/well for 24-well plates. In proliferation studies co-cultures were incubated for 6 days total. For FACS studies involving induction of MHC antigens on endothelial cells by allogeneic T cells co-cultures were incubated for 4 days total.

In some experiments T cells were co-cultured with an endothelial hybrid cell line, EA.hy926 (a gift from C.-J. Edgell, University of North Carolina, NC) (14). The hybrids were originally derived by fusing endothelial cells with the epithelial hybrid cell line A549 obtained from a human lung carcinoma (15). EA.hy926 and A549 cells were grown in medium supplemented with 10% FCS.

In other experiments endothelial cells were deliberately contaminated by low density peripheral blood leucocytes (approximately 30% dendritic cells, 60% monocytes and few T cells; a gift from A. Stagg, Clinical Research Centre, Harrow, UK) prepared as described (16).

Measurement of T cell proliferation

T cell proliferation was measured over the final 18 h of co-culture with EC by addition of 1 μCi/well of [^{3}H]thymidine (Amersham International, Amersham, Bucks, UK) together with 3 μg/ml 5-fluoro-deoxyuridine (Sigma) to prevent synthesis of thymidine by EC (4). Cells were harvested onto a printed filtermat (Wallac Oy, Turka, Finland) using a cell harvester (LKB 12595-001, Pharmacia, Wallac, Sweden) and incorporation of radiolabel was measured using a Pharmacia Wallac 1205 Betaplate automatic microprocessor-controlled liquid scintillation counter (Pharmacia, Wallac, Sweden).

In some co-culture experiments with both CD4⁺ and CD8⁺ T cells together responding to endothelial cells, predicted values were calculated from the individual proliferation of CD4⁺ or CD8⁺ T cells to endothelial cells. The calculation made allowances for the fact that endothelial cells contributed to the counts (despite mitomycin treatment), and to avoid allowing for this twice over the predicted results were calculated as follows

$$(\text{mean EC} + \text{CD4 c.p.m.}) + (\text{mean EC} + \text{CD8 c.p.m.}) - \text{mean EC alone}$$

where means were from three triplicate wells.

FACS analysis of EC for MHC class I and II expression

EC cell surface antigen expression was analysed by indirect immunofluorescence staining (3). Cells were initially incubated with a primary mAb at a saturating concentration of 1/200 followed by a saturating 1/20 dilution of secondary antibody

(FITC-conjugated rabbit anti-mouse IgG, Dakopatts , Denmark). After washing to remove unbound antibody the c were fixed with 1% paraformaldehyde and analysed as descri previously (17) using a Becton-Dickinson FACStar^{PLUS} F Cytometer (Becton-Dickinson, Mountain View, CA) with a argon ion laser (Spectrophysics, Sunnyvale, CA).

Primary mAb used were W6/32 (anti-MHC class I mc morphic determinant; Serotec) and the anti-MHC clas antibodies TAL14.1 (a gift from J. Bodmer, Imperial Car Research Fund Laboratories, London, UK) or L243 (provi by D. Altmann, Transplantation Biology, CRC, Harrow, UI

In some experiments a mAb (NIB42, a gift from A. Mea Division of Immunology, National Institute for Biological Stand and Control, Herts, UK) against IFN-γ was used to block activity of this cytokine. NIB42 had a final neutralization titr $1:5\times10^3$ and was used to a final dilution of 1:50.

Results

Allogeneic CD4+ T cells proliferate in response to IFN-γ-trea EC but not to untreated EC

Figure 1(a) summarizes the results of three experiments to sl the basic proliferative response of allogeneic CD4+ T c co-cultured with EC. There is marked T cell proliferatior IFN-γ-treated EC but the CD4+ cells do not respond to EC have not been pretreated with IFN-γ.

Figure 1(b) shows the results obtained using EC that w deliberately contaminated with 10% low density peripheral bl leucocytes and then sorted by FACS prior to plating. The FA sorting procedure aimed to remove class II or CD45 (comr leucocyte antigen) expressing cells.

There was relative specificity of the response to EC since dermal fibroblasts were unable to stimulate allogeneic CD4+ T cell proliferation when pre-treated with IFN-γ, despite expressing equivalent amounts of MHC class II antigens (data not shown). The proliferative response of CD4+ T cells to IFN-γ-treated EC was strongly inhibited (80.6% of control) by the class II mAb L243, but not by the anti-class I mAb W6/32 (3.8% of control). These data are shown in Table 1.

T cells showed an enhanced proliferative response to the unsorted EC that were contaminated with 10% low density leucocytes which were enriched for dendritic cells and monocytes (16). This was most marked with IFN-γ-untreated cells, where there was a >9-fold increase. There was no response to untreated EC which were contaminated with low density leucocytes and then FACS sorted.

Allogeneic CD4+ T lymphocytes proliferate in response to the hybrid endothelial cell line EA.hy926 when pretreated with IFN-γ

The aim of this set of experiments was to see if an endothelial cell line, devoid of contaminating monocytes, could induce CD4+ T cell proliferation when pretreated with IFN-γ.

Figure 2 shows that allogeneic CD4+ cells proliferate in response to the EA.hy926 cells that have been pretreated with IFN-γ but are unable to respond to the resting unstimulated cells. In contrast, CD4+ cells are unable to respond to fusion partner parent A549 cells regardless of whether or not they have been pretreated with IFN-γ.

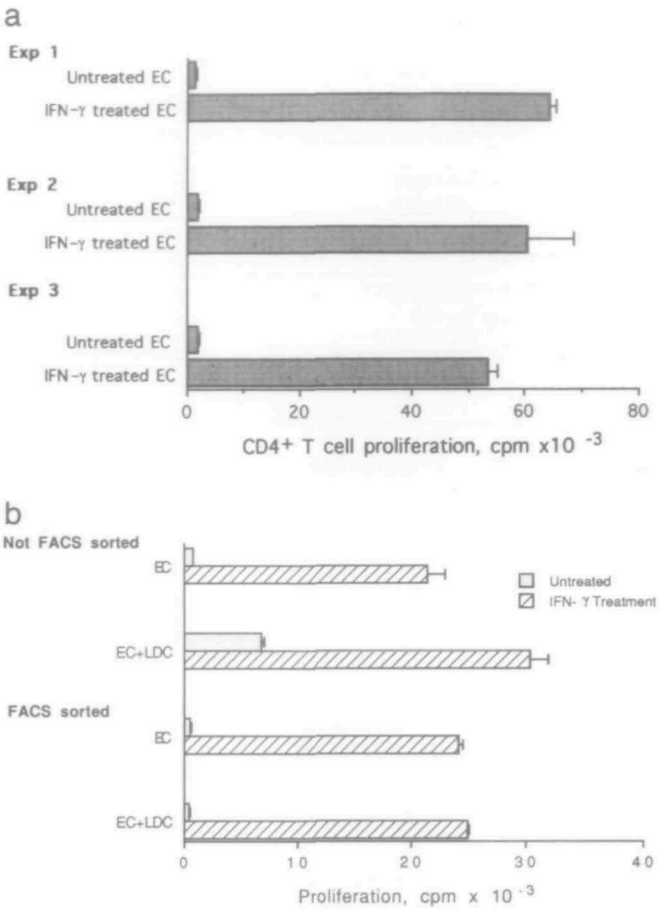

Fig. 1. (a) Proliferation of allogeneic CD4+ T cells in response to IFN-γ-treated EC. T cells alone or EC alone consistently gave $<1.0\times10^{-3}$ c.p.m. (b) Half of an EC population were contaminated with 10% low density leucocytes (LDC) prior to either direct plating or FACS sorting and then plating. Cell populations for FACS sorting were stained with FITC-anti-DR or FITC-anti-CD45 mAb as described in Methods, to remove HLA-DR or CD45 expressing cells. After sorting EC were transferred to 96-well plates, grown to confluent density and, where necessary, treated with IFN-γ before co-culture with CD4+ T cells. T cells alone gave 0.2×10^{-3} ± 0.01 c.p.m., untreated EC gave 0.34×10^{-3} ± 0.02 c.p.m., IFN-γ-treated EC gave 0.9×10^{-3} ± 0.18 c.p.m., untreated EC + LDC gave 1.3×10^{-3} ± 0.08 c.p.m., and IFN-γ-treated EC + LDC gave 1.98×10^{-3} ± 0.12 c.p.m.

Table 1. The involvement of MHC antigens in CD4+ T cell proliferation

Test	Proliferation (c.p.m.)	Inhibition (%)
IFN-γ EC + control mAb	9005 ± 338	
IFN-γ EC + anti-MHC I mAb	8667 ± 194	−4
IFN-γ EC + anti-MHC II mAb	1755 ± 535	−80.5

Antibodies used in this experiment were W6/32 (anti-class I mAb), L243 (anti-class II mAb) and an irrelevant (IgG1) mAb. Each antibody was used at a final well concentration of 20 μg/ml and the antibodies were incubated for the duration of the experiment. Results are shown as the mean of three replicates ± SEM. The antibodies had no effect on untreated EC.

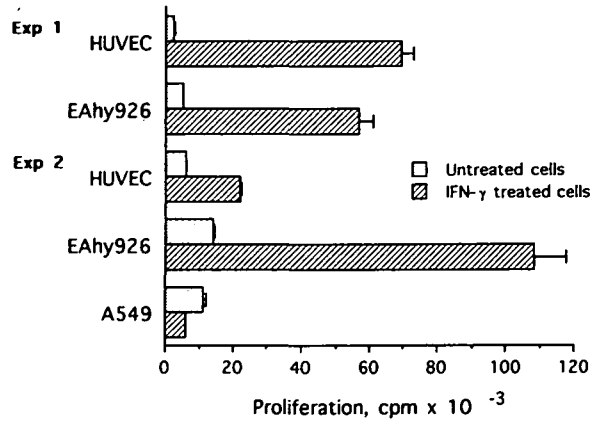

Fig. 2. Results from two experiments showing proliferative responses of CD4+ T lymphocytes to the hybrid endothelial cell line EA.hy926. In the second experiment the ability of A549 cells to stimulate allogeneic CD4+ T cell proliferation was examined. Results are shown as triplicates ± SEM.

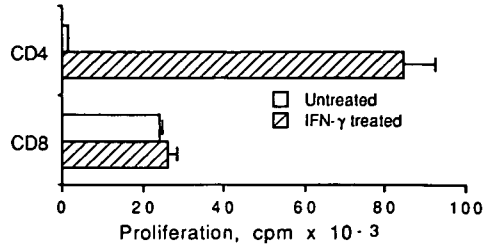

Fig. 3. Proliferation of CD4+ and CD8+ T cells (3×10^5 T cells/well) to untreated and IFN-γ-treated EC. Results are shown as the mean of three replicates ± SEM. T cells alone and EC alone gave $< 0.5 \times 10^{-3}$ c.p.m. and $< 1.2 \times 10^{-3}$ c.p.m., respectively.

Table 2. The involvement of MHC antigens in CD8+ T cell proliferation

Test	*Proliferation* (c.p.m.)	*Inhibition* (%)
IFN-γ EC + control mAb	10,002 ± 999	
IFN-γ EC + anti-MHC I mAb	918 ± 144	−91
IFN-γ EC + anti-MHC II mAb	10,854 ± 114	+8.5

Antibodies used in this experiment were W6/32 (anti-class I mAb), L243 (anti-class II mAb) and an irrelevant (IgG1) mAb. Each antibody was used at a final well concentration of 20 μg/ml and the antibodies were incubated for the duration of the experiment. Similar effects were observed with resting EC (control mAb 9686 ± 1650 c.p.m.; W6/32 780 ± 55 c.p.m.; L243 9820 ± 1721 c.p.m.). Results are shown as the mean of three replicates ± SEM.

Allogeneic CD8+ T cells proliferate in response to resting EC and IFN-γ treated EC

Unlike CD4+ T cells, CD8+ T cells proliferate in response to EC regardless of whether or not they have been stimulated by the cytokine IFN-γ (Fig. 3). The response by CD8+ T cells to IFN-γ-pretreated EC is characteristically much lower than that obtained with CD4+ T cells. The responses of CD8+ T cells to resting or to IFN-γ-treated EC are generally of the same order of magnitude.

The specific involvement of endothelial cell MHC class I antigens was shown by the ability of anti-MHC class I mAb (W6/32) to inhibit the response. By contrast, co-incubation with an anti-class II mAb (L243) had no effect on CD8+ T cell proliferation (Table 2).

Synergistic effects of co-culturing CD4+ and CD8+ T cells with EC

CD4+ and CD8+ T cells show synergistic responses to EC (Fig. 4). Thus when CD4+ cells are 'spiked' with 30% CD8+ cells the response to untreated EC is greater than predicted if the results were additive (see Methods for calculation). The response to IFN-γ-treated EC appears to be maximal and is not further increased by CD8+ cells. When CD8+ cells are spiked with 30% CD4+ cells there is a greater response to both untreated and to IFN-γ-treated EC than would be predicted. A similar pattern was seen if only 10% of CD4+ or CD8+ cells were mixed with the opposite subset or if cells were mixed in equal proportions.

The synergistic response of co-culturing CD4+ and CD8+ T cells with EC is due to CD4+ proliferation

The synergistic response obtained when co-culturing CD4+ and CD8+ T cells with EC was examined by treating one of the T cell subsets with mitomycin C so that proliferation of the other allogeneic T cell subset could be studied. Under these conditions it was found that CD4+ and CD8+ T cells show a synergistic proliferative response when the CD8+ T cell subset had been treated with mitomycin C but not if the CD4+ T cell subset had been treated with mitomycin C (Fig. 5), suggesting that the CD4+ T cell subset contributed the most to the synergism.

Synergy cannot be reconstituted by conditioned media

The proliferative response of CD4+ T cells (or indeed of CD8+ T cells) to untreated EC is not enhanced by conditioned media obtained from EC (untreated) co-cultured with allogeneic CD4+ or CD8+ T cells (Fig. 6).

Induction of EC MHC class II antigens by CD8+ or CD4+/CD8+ T cells

The effects of CD4+ or CD8+ T cells—alone or in combination—on expression of MHC molecules by EC is shown in Fig. 7. IFN-γ upregulates MHC class I expression and induces EC class II expression. CD4+ T cells upregulate EC class I antigens but not class II antigens. Allogeneic CD8+ T cells also upregulate MHC class I expression as do a mixture of CD4+ and CD8+ T cells. In contrast to CD4+ T cells, CD8+ T cells induce EC MHC class II expression.

Depletion of CD8+ T cells (or CD16+ T cells) from PBL also reduced the ability of a PBL population to induce class I or class II antigens (data not shown). Depletion of CD4+ or CD19+ cells had no effect. The induction of both class I and class II antigens in these responses were fully inhibitable by mAb against IFN-γ.

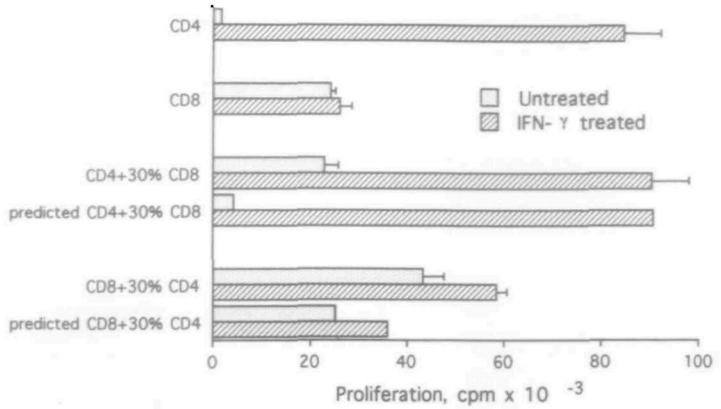

Fig. 4. The effect of co-culturing $CD4^+/CD8^+$ T cells, alone or in combination, with EC. EC were co-cultured with 3×10^5 T cells unless indicated by 30% $CD4^+$ or $CD8^+$ (i.e. 9×10^4 T cells). Results are shown as the mean of three replicates ± SEM. Not shown on the graph are the following results: 30% $CD8^+$ T cells gave 2.7×10^{-3} ± 0.19 c.p.m. with untreated EC and 6.0×10^{-3} ± 0.99 c.p.m. with IFN-γ-treated EC. 30% $CD4^+$ T cells gave 0.9×10^{-3} ± 0.03 c.p.m. with untreated EC and 9.9×10^{-3} ± 3.7 c.p.m. with IFN-γ-treated EC. EC alone gave 0.9×10^{-3} ± 0.06 c.p.m. (when untreated) and 1.3×10^{-3} ± 0.09 c.p.m. (when IFN-γ-treated). Predicted results were calculated as described in Methods, allowing for any duplication of counts from EC alone. The experiment was repeated five times.

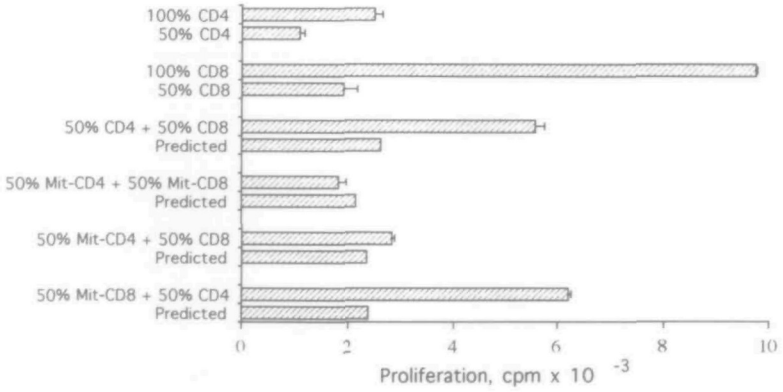

Fig. 5. The effects of treating one T cell subset with mitomycin C on the synergy of $CD4^+$ and $CD8^+$ T cells co-cultured with resting EC (not IFN-γ-treated). In this figure 3×10^5 T cells are indicated by 100%. T cells were treated for 60 min with 10 μg/ml mitomycin C in Hank's buffered saline solution before being washed and used in the experiment. Results are shown as the mean of three replicates ± SEM. Not shown on the graph are the following results: 50% mitomycin C-treated $CD4^+$ T cells gave 0.86×10^{-3} ± 0.07 c.p.m., 50% mitomycin C-treated $CD8^+$ T cells gave 1.7×10^{-3} ± 0.02 c.p.m., and resting EC alone gave 0.4×10^{-3} ± 0.01 c.p.m.

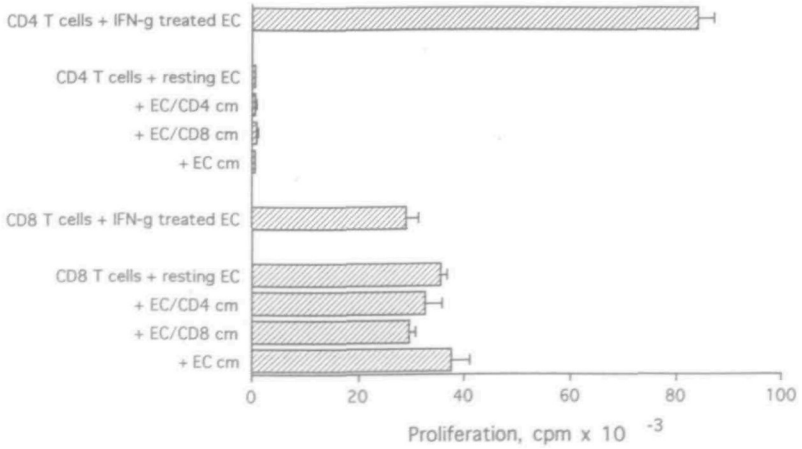

Fig. 6. The effect of conditioned media (cm) (as 50% of the 200 μl volume present per well) on T cell proliferation. Conditioned media were supernatants obtained from co-culturing T cells with resting EC (EC/CD4 cm; EC/CD8 cm) or from EC alone (EC cm). These supernatants were stored at −20°C and filtered before use. Results are shown as the mean of three replicates ± SEM.

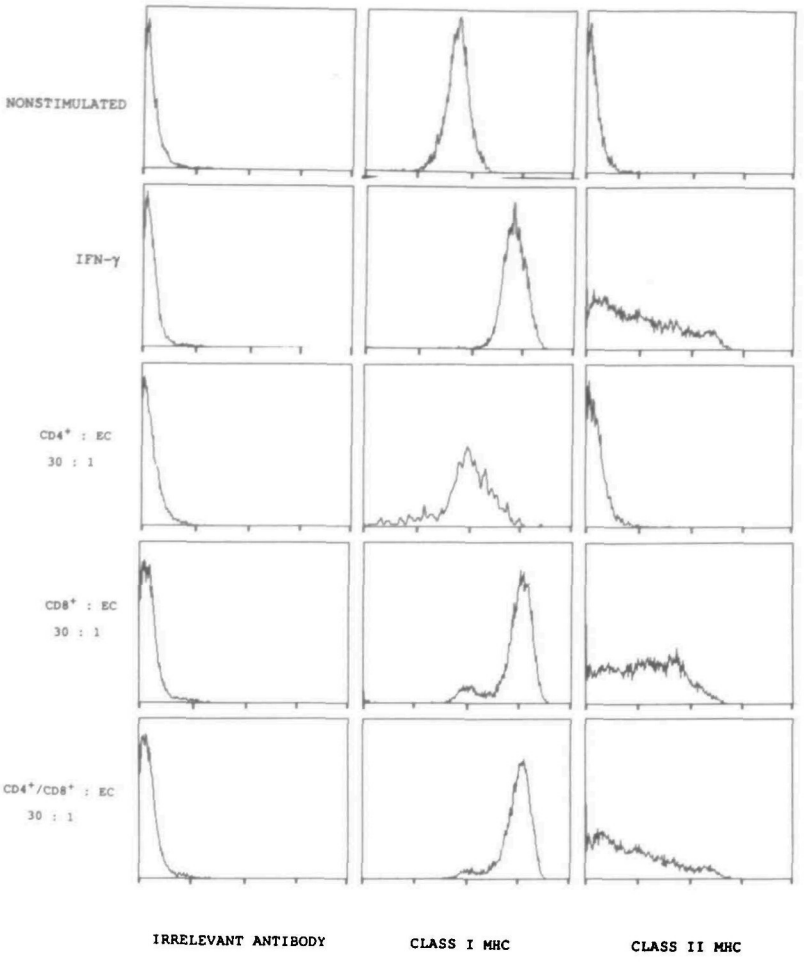

Fig. 7. The effect on EC MHC expression of co-culturing $CD4^+$ or $CD8^+$ T cells with vascular EC at the ratios indicated.

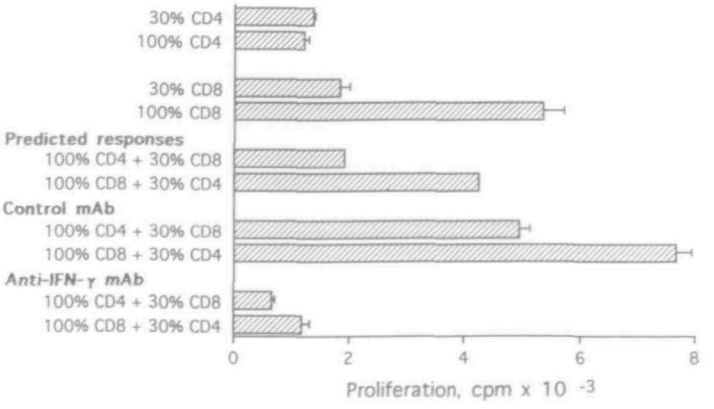

Fig. 8. The effect of the IFN-γ mAb NIB42 on the synergism of $CD4^+/CD8^+$ T cells co-cultured with resting EC. NIB42 (1 in 50 dilution) and a control mAb (12 μg/ml) were included in the relevant wells for the duration of the experiment from the start of co-incubation of EC with $CD4^+/CD8^+$ T cells. AFter 36 h further antibodies were added to the relevant wells. Results are shown as triplicates ± SEM. Resting EC alone gave 0.74×10^{-3} ± 0.06 c.p.m., 100% $CD4^+$ or $CD8^+$ T cells responding to IFN-γ-treated EC gave 42.5×10^{-3} ± 0.04 c.p.m. and 24.9×10^{-3} ± 0.74 c.p.m. respectively, while $CD4^+$ or $CD8^+$ T cells alone gave 0.18×10^{-3} ± 0.005 c.p.m. and 0.12×10^{-3} ± 0.02 c.p.m. respectively.

Proliferation of CD8+ T cells in response to cultured EC can be inhibited by the IFN-γ mAb NIB42

The synergy between CD4+ and CD8+ T cells when co-cultured with vascular EC is inhibited by the IFN-γ mAb NIB42 used at a dilution of 1 in 50 (Fig. 8). A control isotype-matched mAb had no effect on the synergistic response of CD4+/CD8+ T cells co-cultured with vascular endothelium.

Discussion

In this study we have compared the interaction of purified populations of CD4+ and CD8+ T cell subsets with pure populations of human large vein EC obtained from umbilical cords. The critical baseline response for these studies is the ability of highly purified CD4+ T cells to proliferate in response to MHC class II molecules expressed by EC that have been pretreated with IFN-γ but not to untreated and MHC class II antigen-negative EC (3). As previously described, this response is highly dependent on the purity of the CD4+ T cell populations. Indeed, neither the CD4+ T cell population nor the EC cell population contain class II expressing cells as determined by reverse transcriptase–polymerase chain reaction analysis (for both populations) and by lack of ability of the CD4+ T cells to respond to the superantigen staphylococcal enterotoxin B. Further evidence that EC can induce allogeneic T cells to proliferate was provided by studies using the hybrid EC line EA.hy926. Interestingly, fibroblasts (resting or pretreated with IFN-γ) are unable to stimulate CD4+ T cell proliferation, confirming earlier work by Geppert and Lipsky (18).

Unlike CD4+ T cells, CD8+ T cells are able to proliferate in response to resting EC and this response can be blocked by using mAb to MHC class I antigens. The proliferative response of allogeneic CD8+ T cells could not be significantly upregulated by pretreatment of EC with IFN-γ, despite being able to detect significant upregulation of EC MHC class I expression by FACS. Thus the CD8+ T cell population appeared to respond maximally to the constitutively expressed MHC class I molecules. Recently Adams *et al.* (19) have reported that IL-2 secretion by CD8+ T cells stimulated with resting EC could not be enhanced by pretreatment of EC with IFN-γ.

Combined populations of CD4+ and CD8+ T cells show a synergistic alloproliferative response when co-cultured with EC. The synergism appears to be due to the ability of CD4+ T cells to respond to MHC class II antigens, since mitomycin C treatment of CD4+ T cells abolished the synergistic response while mitomycin C treatment of the CD8+ subset did not. Thus CD8+ T cells appeared to stimulate the proliferation of CD4+ T cells in response to resting EC. To determine whether a soluble factor secreted by T cells was responsible, conditioned media were tested, but were ineffective. Next we were interested in earlier reports that CD8+ T cells could induce class II expression on EC (which CD4+ might recognize and respond to) by the secretion of IFN-γ (17,20); we were aware that IFN-γ has a short half-life in tissue culture media, suggesting that an IFN-γ-mediated effect might have been missed by using conditioned media. Initial experiments to see whether CD8+ T cells and fixed EC were effective in stimulating CD4+ T cells were non-informative because the fixation destroyed the ability of even the CD8+ T cells to respond to the class I alloantigens, as previously described (21). Experiments to show that CD8+ T cells could induced MHC class I and class II antigens confirmed previous reports (17,18), and anti-IFN-γ mAb was able to block the induction of class II antigens both by recombinant IFN-γ and by CD8+ T cells. Furthermore, anti-IFN-γ antibody also blocked the synergistic response between CD4+ and CD8+ T cells to EC, indicating that CD8+ T cells are able to induce EC MHC class II antigens by secreting IFN-γ, to which class II antigens the CD4+ T cells can then respond.

Interestingly, the proliferative response of 3×10^5 CD4+ T cells to recombinant IFN-γ-pretreated EC was generally greater than with the CD4/8 mixtures, despite the fact that a 30:1 ratio of CD8+ T cells:EC, a similar ratio to that present in a 96-well plate when 3×10^5 T cells are added, appeared to induce similar levels of MHC class II as pretreatment of EC with IFN-γ (see Fig. 7). There are two reasons for this. First, we were unable to use both 3×10^5 CD8+ T cells (to induce class II) and 3×10^5 CD4+ T cells (to make the proliferative response) in a single well and maintain reasonable cell viability. Second, the MHC class II induced by recombinant IFN-γ was already present when the CD4+ T cells were added, while that expressed in response to CD8+ T cells still had to be induced.

On the basis of our findings we suggest that both CD8+ and CD4+ T cells are involved in allogeneic responses of T cells to EC, since CD8+ T cells respond to constitutively expressed MHC class I antigens and induce MHC class II antigens which CD4+ T cells then respond to. These findings suggest that EC may initiate allogeneic reactions by both CD8+ and CD4+ T cell subsets. These interactions may be of importance in organ allograft rejection, particularly between a DR-mismatched donor and recipient.

Acknowledgements

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Abbreviations

EC	endothelial cell
HLA	human leucocyte antigen
LFA-3	lymphocyte function antigen-3
PBL	peripheral blood lymphocytes

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