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Eicosanoids are regulatory molecules in γ-interferon-induced endothelial antigenicity and adherence for leucocytes

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When the endothelial cells (ECs) were stimulated with γ-interferon (gIFN) in the presence of methylprednisolone (MP) or prostaglandin E_2 (PGE_2), MP enhanced gIFN-induced Ia antigen expression, whereas PGE_2 inhibited it. On the other hand, while PGE_2 had no effect on leucocyte binding to ECs, MP entirely inhibited it. By using selective inhibitors of the cyclo-oxygenase pathway (indomethacin, IM) and the 5-lipoxygenase pathway (L651.392), we found that addition of IM to gIFN-stimulated ECs enhanced Ia expression but had no effect on leucocyte adherence to ECs. Instead, addition of L651.392 to gIFN-stimulated ECs partially reduced leucocyte adherence to ECs but had no effect on Ia expression. Pretreatment of the ECs or leucocytes or both with monoclonal anti-class II antibody, had no effect on gIFN-induced leucocyte binding to ECs. These findings suggest that gIFN-induced endothelial cell antigenicity and leucocyte adherence are regulated independently of each other by different molecular pathways. Moreover, arachidonic acid metabolites appear to be the regulatory molecules in gIFN effects on the ECs.

Endothelium antigenicity; Leukocyte-endothelial adhesion; Eicosanoid

1. INTRODUCTION

Graft endothelial cells (ECs) play an important role in the initiation and maintenance of transplant rejection: (i) they are the first cells to come into contact with host leucocytes; (ii) they either express or may be induced to express class II MHC (Ia) antigens [1] and act as antigen presenting cells [2]; (iii) they possess also class I MHC antigens (as all nucleated cells do) and therefore are obvious targets for host cytotoxic T cells, and (iv) they regulate traffic of inflammatory leucocytes from blood stream into graft parenchyma [3]. Factors responisble for upregulation of endothelial antigen expression and permeability for leucocytes are inflammatory cytokines, secreted by cells at the site of inflammation. Since ECs are not replaced by host cells [4], they are potential inducers of rejection, ad infinitum.

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In this communication we demonstrate, by using selective inhibitors of eicosanoid synthesis, that two effects of γ-interferon (gIFN) on rat ECs, the enhancement of Ia antigen expression and leucocyte adhence, are regulated independently of each other and are most likely influenced by intermediary molecules, eicosanoids.

2. MATERIALS AND METHODS

2.1. *Rats*

The nuclei of colonies of the inbred rat strains Wistar Furth (WF; AgB-2, RT1u) and DA (AgB-4, RT1a) were obtained from Professors O. Sjögren (University of Lund, Lund, Sweden) and J. Gowans (Dunn School of Pathology, Oxford, England), respectively.

2.2. *Endothelial cell cultures*

Endothelial cells were isolated from the hearts of 7–10 days old DA rats using a modification of the method of Kasten et al. [5,6]. Purity of the EC population was evaluated by indirect immunoperoxidase staining with antibodies against factor VIII related antigen (FVIIIr:ag) and leucocyte contamination examined with antibody to leucocyte common antigen (LCA).

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Cultures exceeding 99% of FVIIIr:ag positive cells and no detectable LCA expressing cells were used.

2.3. *Antibodies*

Mouse monoclonal antibodies against rat class II MHC antigen (clone OX4) and against rat LCA (clone OX1) were from Sera-Lab (Crawley Down, Sussex, England). Rat monoclonal antibody against mouse LFA-1 molecule (clone H35.89.9 [7]), cross-reacting with rat LFA-1, was a gift from Dr M. Pierres (CNRS, Marseille, France). Polyclonal antibodies: rabbit anti-mouse IgG conjugated with peroxidase and rabbit anti-human FVIIr:ag (cross-reacts with rat FVIIIr:ag) were obtained from DAKO (Copenhagen, Denmark). Goat polyclonal antibody to rabbit IgG conjugated with peroxidase was from Tago Inc. (Burlingame, CA, USA).

2.4. *Compounds for EC treatment*

Recombinant rat γ-interferon was a gift from Dr P.H. van de Meide and Dr H. Schellekens (Primate Centre, TNO, Rijswijk, The Netherlands). L651.392, an inhibitor of 5-lipoxygenase [8], was a gift from Dr A.W. Ford-Hutchison (Merck-Frost, Canada). Prostaglandin E_2 (PGE_2) and arachidonic acid (AA) were from Upjohn (Calamazoo, MI, USA). Solu-Medrol (methylprednisolone) was purchased from Upjohn (Puurs, Belgium).

2.5. *Indirect immunoperoxidase staining*

After 3–5 days of culture in bottles, ECs were plated in chamber/slides (4 chambers per slide; Miles Scientific, Naperville, IL, USA) and treated with different compounds for 3 days. After treatment cells were fixed in situ with 5% paraformaldehyde and labelled with mouse monoclonal or rabbit polyclonal antibody, followed by one or two peroxidase conjugated polyclonal antibodies (rabbit anti-mouse IgG and goat anti-rabbit IgG).

2.6. *Leucocyte binding to EC*

Binding assay was performed following the modified method of Butcher et al. [9]. Compared to the original method, rotation of specimens during incubation of ECs with freshly isolated spleen cells of WF rat was omitted, because in our experiments it did not have an effect on leucocyte binding to ECs.

3. RESULTS

Steroids (methylprednisolone; MP) are potent inhibitors of eicosanoid synthesis. In the first experiment we investigated the effect of gIFN on endothelial cell antigenicity and leucocyte adhesion to ECs in the presence or absence of therapeutic quantities (1 μg/ml) of MP. For control we used PGE_2, known from previous studies to inhibit antigen expression on macrophages [10].

γ-IFN induced, as expected, potent class II MHC expression on ECs and, concomitantly, ECs become adherent to allogenic leucocytes (table 1). The effect was dose dependent (not shown). At a concentration of 1 μg/ml, MP entirely inhibited gIFN-induced leucocyte adhesion to ECs, but unexpectedly, significantly enhanced endothelial expression of Ia antigen. On the other hand, at 10^{-7} M, PGE_2 reduced gIFN-induced class II MHC expression but had no effect on leucocyte-endothelial adhesion (table 1). Thus, this experiment demonstrated that prostaglandins and eicosanoid inhibitors can modify gIFN effects on endothelium. Secondly, the experiment suggested that endothelial Ia expression and adhesiveness for leucocytes, when triggered by gIFN, are regulated by different molecular pathways and not interdependent.

Table 1

Effect of inhibition of endothelial eicosanoid synthesis on expression of Ia-antigen and leucocyte-endothelial interaction in vitro

EC treatment[a]	% of Ia positive ECs (mean ± SD)	% of leucocytes binding ECs (mean ± SD)
Nil	8.0 ± 1.0	2.3 ± 0.6
gIFN[b]	50.7 ± 2.1***	20.0 ± 1.0***
MP	12.7 ± 3.1	1.0 ± 0.0
IM	11.3 ± 1.1	1.3 ± 0.6
L651.392	7.0 ± 1.0	1.0 ± 0.6
PGE_2	7.0 ± 1.0	1.0 ± 0.0
AA	7.0 ± 0.0	2.3 ± 1.5
gIFN + MP[c]	75.7 ± 1.5***	2.7 ± 1.5***
gIFN + IM[c]	69.7 ± 1.5***	17.3 ± 2.1 ns
gIFN + L651.392[c]	52.3 ± 4.0 ns	10.7 ± 2.1**
gIFN + PGE_2[c]	36.3 ± 1.5***	20.3 ± 2.5 ns
gIFN + AA[c]	49.3 ± 2.1 ns	20.1 ± 2.5 ns
gIFN + MP + AA[c]	39.3 ± 3.1**	17.7 ± 2.1 ns
gIFN + IM + AA[c]	66.0 ± 2.6**	19.0 ± 2.6 ns
gIFN + L651.392 + AA[c]	52.3 ± 3.5 ns	10.3 ± 3.2*

[a] Concentrations: gIFN, 100 U/ml; MP, IM, L651.392, 1 μg/ml; PGE_2, AA, 10^{-7} M

[b] Significancies versus control (line 1)

[c] Significancies versus gIFN treatment alone (line 2)

Significancies in Student's t-test: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.1$; ns, $p > 0.1$ (not significant)

In the second experiment we employed gIFN stimulation and selective inhibition for the cyclooxygenase (CO) or 5-lipoxygenase (LO) pathways of eicosanoid synthesis, or both, and free arachidonic acid (AA).

MP, indomethacin (IM, inhibitor of CO), L651.392 (inhibitor of LO), and AA did not alter

Table 2

Effect of pretreatment of cells with monoclonal antibody on leucocyte adherence to endothelium

EC stimulation	Antibody	Treatment with antibody[a]		% of ECs interacting with leucocytes
		Endothelium	Leucocytes	
Nil	OX4	–	–	1–2
Nil	OX4	+	–	1–2
Nil	OX4	–	+	1–2
Nil	OX4	+	+	1–2
gIFN	OX4	–	–	37–69
gIFN	OX4	+	–	40–61
gIFN	OX4	–	+	35–38
gIFN	OX4	+	+	35–58
gIFN	H35-89.9	–	+	1–2

[a] Treatment with antibody was performed for 30 min at room temperature. Antibody concentration was 0.2 mg/ml

the background endothelial cell Ia expression and/or endothelial cell adherence with leucocytes (table 1). When added together with gIFN, MP and IM enhanced gIFN-induced Ia expression, but L651.392 had no effect. On the other hand, MP and L651.392 reduced gIFN-induced leucocyte adherence to ECs, but IM had no effect. Finally, when the same inhibitors were added together with gIFN and AA, extraneous AA was able to overcome the MP effect, but not the effects of IM and L651.392. Thus these experiments confirmed that eicosanoids are involved in gIFN-induced effects on endothelial cells, acting possibly as intermediary (regulatory) molecules, and that whilst the leucocyte adhesion to endothelium is regulated by the LO pathway, the CO pathway seems to be involved in endothelial antigenicity.

Finally, we wished to formally demonstrate that the gIFN effects on endothelial Ia expression and leucocyte adhesion to endothelium are independent of each other, by attempting to inhibit the leucocyte adhesion to endothelium with anti-class II MHC antigen antibody. The results are shown in table 2. Treatment of the endothelial cells, or the leucocytes or both with antibody OX4 did not alter the leucocyte adhesion to the endothelium. Instead, treatment of leucocytes with antibody to LFA-1 molecule (control), entirely inhibited the adherence.

4. DISCUSSION

Our results suggest that the effects of gIFN on endothelial cell Ia expression and on the binding of allogeneic leucocytes to endothelium are regulated by intermediary molecules, eicosanoids. Products of the cyclo-oxygenase pathway appear to be involved in the regulation of endothelial class II MHC antigen expression, whereas products of the lipoxygenase pathway seem to regulate the adherence of leucocytes to endothelium. Steroids completely block production of eicosanoids by inhibiting the action of phospholipase A_2 responsible for releasing free arachidonic acid from its cell membrane phospholipid-bound form [11]. Therefore, enhancing the effect of MP on Ia expression appears to be related to the abolition of prostaglandin production by the endothelium, which seems to function as a 'natural' downregulatory system for the Ia expression on ECs. This conclusion is supported by the finding that PGE_2 when added to EC cultures together with gIFN, had an inhibitory effect on class II MHC expression. IM, which blocks the production of prostaglandins by interfering with cyclo-oxygenase activity, had an MP-like effect on Ia expression.

In addition to the effect on Ia expression, steroids also appear to affect leucocyte-endothelial adherence. The most probable reason is the blocking of lipoxygenase pathways, by cutting off free arachidonic acid supply. This suggestion is confirmed by the suppressive effect of the 5-lipoxygenase inhibitor, L561.392, on leucocyte binding to ECs, and restoration by AA when added together with MP. Incomplete inhibition of binding by L651.392, compared to MP, is caused most likely by the presence of other lipoxygenase pathways in ECs (e.g. 15-lipoxygenase pathway), which can produce other binding-stimulating mediators (e.g. 15-HETE) [12]. Leukotriene B_4, a product of the 5-lipoxygenase pathway, has already been shown to enhance the adherence of leucocytes to endothelial cells [13].

Finally, in contradiction to the results of Masuyama et al. [14], we did not find Ia antigen to be involved in leucocyte binding to the endothelium. Regulation of Ia expression, by different mediators or blocking of Ia antigen with monoclonal antibody, did not have any effect on

leucocyte-endothelial interaction. For comparison, treatment of leucocytes with monoclonal antibody against LFA-1 molecule [15] showed complete inhibition of leucocyte-endothelial binding.

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