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Leukotriene B_4 increases the lymphocyte binding to endothelial cells

Risto Renkonen, Pirkko Mattila, Dariusz Leszczynski and Pekka Häyry

Transplantation Laboratory, University of Helsinki, Helsinki, Finland

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Leukotrienes are potent mediators of local microvascular environment. Leukotriene B_4 treatment of cultured endothelium increases the binding of lymphocytes to endothelial cell monolayers within minutes. This effect is dose-dependent and reversible upon removal of the leukotriene. Pretreatment of lymphocytes slightly decreases the binding and pretreatment of both lymphocytes and endothelium with leukotriene B_4 prior to the adherence assay did not alter the binding. These results suggest that leukotriene B_4 regulates exclusively the vascular side, but not the white cell side of this interaction.

Leukotriene B_4; Endothelial cell; Lymphocyte homing

1. INTRODUCTION

Leukotrienes B_4, C_4 and D_4 (LTB_4, LTC_4, LTD_4) all augment vasopermeability after intradermal injection in both humans and other animals [1,2]. LTB_4 increases granulocyte adherence to the microvascular endothelium in the hamster cheek pouch [3,4], and granulocyte extravasation after intradermal injection [2,5]. Increased adherence of granulocytes has been demonstrated after in vitro treatment of endothelial cell monolayers with LTB_4 [6].

Allograft rejection is most likely regulated at the level of the microvascular endothelial cells (EC), as this is the site of entry of inflammatory white cells into the graft [7]. During the initial phase of rejection, the allograft is infiltrated predominantly by lymphocytes and monocytes [8]. Since LTB_4 increases granulocyte adhesion to EC monolayers in vitro, we tested the effect of LTB_4 on the lymphocyte-EC interaction.

Incubation of EC with LTB_4 increased lymphocyte binding to EC in a dose-dependent manner. The effect was very rapid, and reversible after removal of the leukotriene. On the other hand, the LTB_4 incubation of lymphocytes slightly decreased the binding, and if both the cell types were LTB_4 treated no alteration in the binding was seen.

Correspondence address: R. Renkonen, Transplantation Laboratory, University of Helsinki, Haartmaninkatu 3 A, 00290 Helsinki, Finland

2. MATERIALS AND METHODS

2.1. *Isolation and culture of endothelial cells*

4–10-day-old DA rat hearts were minced with a scalpel and incubated three times in serum-free MEM with 0.2 mg/ml of DNase (600 IU/mg, Sigma, St. Louis) and 0.2 mg/ml of collagenase (183 U/mg, Worthington). After the first incubation (10 min at 37°C, magnetic stirring) the supernatant was discarded. Two more 15-min incubations were performed and the supernatants were collected and filtered through a 50 μm steel mesh. The red blood cells were lysed with lysing reagent for 5 min in 37°C followed by two washings with MEM. The single cells were plated in T-25 tissue culture flasks. After 90 min incubation the non-attached myocardial cells were discarded and the highly adherent endothelial cells were left in the flask. Endothelial cells usually grew to confluence in 5 days and the cells were used at 2–4 passages.

2.2. *Preparation of lymphocytes*

8–10-week-old DA rat spleens were isolated, a single cell suspension was made mechanically and clumps were sedimented for 10 min. Red blood cells were lysed with lysing reagent and the cells were washed twice with MEM. Approx. 20×10^6 lymphocytes were suspended in 0.3 ml MEM and 0.3 ml chromium was added (containing approx. 300 μCi $Na_2{}^{51}CrO_4$, Amersham

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International, Amersham, England). After 2 h incubation at 37°C the cells were washed three times with MEM. Cell viability was always greater than 95%.

2.3. *Lymphocyte to EC monolayer adhesion assay*

Endothelial cells were removed from culture flasks with versene (1 : 5000, Gibco) and trypsin (0.1%, Sigma), centrifuged and suspended in MEM at a concentration of 2×10^5/ml. 0.1-ml aliquots were then cultured overnight in flat-bottomed microwell plates (Nunc) precoated with collagen (Gibco). After the endothelial monolayer was confirmed with an inverted microscope, the EC were washed once, treated with given amounts of LTB_4 for defined time periods and washed with MEM. The ^{51}Cr-labelled lymphocytes (10^5 cells in 0.1 ml) were added to each microwell. After 60 min incubation at 37°C, non-adherent lymphocytes were removed by washing with MEM. The bound lymphocytes were lysed by adding 0.1 ml Triton X-100 (Sigma) to each microwell and the radioactivity of the lysate was measured. The percentage of bound lymphocytes was calculated as follows:

$$\% \text{ of bound lymphocytes} = \frac{\text{cpm in the lysate}}{\text{cpm in the input}} \times 100$$

Each test was performed as triplicates. Data are expressed as a binding index (mean ± SD) where the binding of lymphocytes with LTB_4 is divided by the binding of lymphocytes to untreated EC.

2.4. *Reagents*

LTB_4 was purchased from Upjohn (Michigan, USA).

3. RESULTS

In the first experiment LTB_4 was added at concentrations of 10^{-6}–10^{-9} M to the EC culture just before plating the ^{51}Cr-labelled lymphocytes onto the endothelial cells. With normal, untreated EC, the binding of lymphocytes was 18.0 ± 2.2% of the input population. The addition of LTB_4 resulted in a dose-dependent increase in lymphocyte binding. Maximal effect was observed at 10^{-8} M LTB_4, where 33.4 ± 3.5% of the input population was recovered, leading to a binding index of 1.8 ± 0.15 (fig.1).

In the second experiment either endothelial cells or lymphocytes were incubated separately with LTB_4 for 1, 5 or 15 min, washed and added to the binding assay. EC pretreatment strongly increased the binding with 1 min treatment (binding index 1.73 ± 0.07 at optimal concentration, 10^{-8} M, of LTB_4). A similar effect was noted at all time periods tested (binding index always above 1.5, fig.2). Pretreatment of lymphocytes with LTB_4 slightly decreased the binding on EC (binding index 0.84 ± 0.14). When both the EC and lymphocytes were pretreated for 5 min with LTB_4, washed, and tested in a binding assay, the binding index amounted to 1.0 ± 0.15, i.e. the inhibitory effect of LTB_4 on lymphocytes overcame the enhancing effect of LTB_4 on the EC.

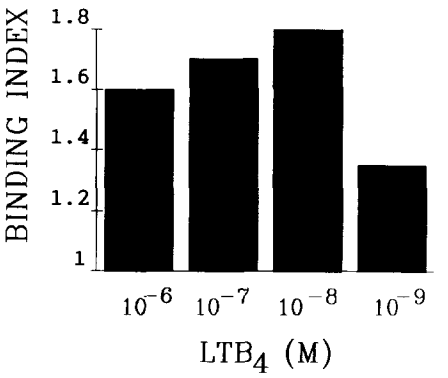

Fig.1. LTB_4 increases lymphocyte binding to endothelial cell (EC) monolayers in a dose-dependent manner. LTB_4 was added to EC cultures at different concentrations just before lymphocytes were added to the assay. Data are expressed as a binding index, where binding of lymphocytes to LTB_4-treated EC is divided by that to untreated EC. Binding of lymphocytes to control EC in the absence of LTB_4 was 18.2 ± 2.2% of input population. Data are pooled from 4 different experiments.

To investigate the duration of the LTB_4-induced effect, the EC were finally treated with 10^{-8} M LTB_4 for 5 min, washed twice with culture medium and lymphocytes were added to the assay after different time periods. The LTB_4-induced, increased binding capacity of EC decreased 30 min after the removal of the leukotriene (binding index

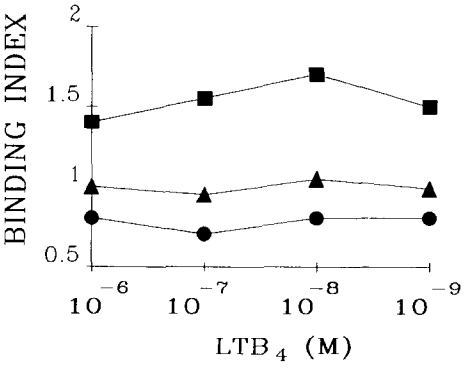

Fig.2. Either EC (■), lymphocytes (●), or both cell types (▲) were pretreated with 10^{-8} M LTB_4 for 5 min and washed twice before assay. For binding index, see legend to fig.1.

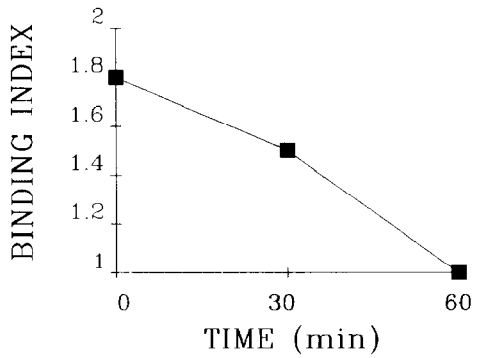

Fig.3. LTB_4-induced lymphocyte binding effect is reversible after removal of the leukotriene. The EC were treated with LTB_4 (10^{-8} M) for 5 min, washed twice and lymphocytes were added to the culture after indicated time periods. For binding index, see legend to fig.1.

1.51 ± 0.1 vs 1.8 ± 0.15) and was totally abolished at 60 min (fig.3).

4. DISCUSSION

Circulating lymphocytes adhere to specialized post-capillary venules of lymphoid tissue (high endothelial venules), lined by a characteristic 'high' endothelium [9]. Lymphocytes adhere to the endothelium only for a few seconds followed either by their penetration through the endothelial layer into the lymphoid tissue or detachment and continuation of circulation [10]. The in vitro lymphocyte-endothelial cell monolayer assay has been used extensively in studies of lymphocyte migration. Incubation of the EC with γ-interferon increases the lymphocyte binding significantly within days [11]. Incubation with interleukin 1 increases the lymphocyte binding within hours [12]. Pretreatment of the EC with LTC_4 or LTD_4 increases the granulocyte binding within 10 min [13], as does pretreatment with platelet-activating factor (PAF) [13].

Our results demonstrate that incubation of EC with LTB_4 increased lymphocyte binding within minutes. LTB_4 has also been shown to increase granulocyte adherence to EC in vitro [6]. LTB_4 also rapidly increases vascular permeability, measured with fluorescence-labelled dextran in a hamster cheek pouch model [4]. LTB_4 possesses chemotactic and chemokinetic properties [14] and it increases leukocyte influx into the skin, when injected topically intradermally [2].

The effect of LTB_4 on lymphocyte binding to the EC is rapid and transient. EC was incubated with an optimal concentration of LTB_4, followed by washing, and binding was determined after different time periods. At 30 min there was a significant decrease in the binding and at 60 min the increased binding was totally abolished.

Allograft rejection is characterized by an influx of inflammatory white cells into the graft. During the first 4 days of drug unmodified rejection, the majority of the infiltrating cells are lymphocytes and mononuclear phagocytes. Thereafter, when necrotic changes become dominant, the number of granulocytes also increases [8].

Evidence exists that allograft inflammation is regulated locally by different potent, short-lived mediators, i.e. cytokines and eicosanoids, etc. This might explain why, for example combined heart-lung transplants are frequently rejected independently of each other [15]. Leukotrienes have been called 'pro-rejection' eicosanoids [16]. LTB_4 is found in 50-fold greater concentrations in a rejecting kidney allograft cortex [17]. Inflammatory white cells isolated from 'sponge matrix' allografts produce 10-times more LTB_4 than white cells in the control sponges [18]. Preliminary data suggest that the secretion of LTD_4 during rejection into urine also increases [19].

The source of LTB_4 in the allograft can be either inflammatory white cells and/or the graft parenchymal components. Granulocytes, monocytes and eosinophiles produce LTB_4 [20]. In contrast, recently published data demonstrate that highly purified human lymphocytes do not produce detectable amounts of any leukotrienes, analysed by high-performance liquid chromatography and radioimmunoassay techniques [21]. Kidney parenchymal cells including glomerular mesangial and epithelial cells as well as tubular cells show cyclooxygenase and 5-lipoxygenase activity [22]. Interleukin 1 stimulus can upregulate at least the prostanoid production (PGE_2, TxB_2 and 6-keto PGF_{1a}) in mesangial cells [23].

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