Microbial Pathogenesis 113 (2017) 427–431

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

NFκB transcription factor (p65) immunohistochemistry in leprosy dermal microvasculature

Luciana Mota Silva[a], Kelly Emi Hirai[a], Jorge Rodrigues de Sousa[b], Juarez de Souza[a], Leônidas Braga Dias Jr.[a], Francisca Regina Oliveira Carneiro[a], Tinara Leila de Souza Aarão[a], Hellen Thais Fuzii[b], Juarez Antonio Simões Quaresma[a,b,*]

[a] Center of Biological and Health Science, State University of Para, Belem, Brazil
[b] Tropical Medicine Center, Federal do Para University, Belem, Brazil

ARTICLE INFO

Keywords:
Leprosy
Endothelial adhesion molecules
NFκB

ABSTRACT

Leprosy caused by *Mycobacterium leprae* is characterized by a spectrum of clinical manifestations that are determined by the predominant immunological profile of the host. The recruitment of leukocytes to the sites of injury can influence the development of these profiles. Cell adhesion molecules such as ICAM-1, VCAM-1 and CD62E participate in this process and their expression is regulated by transcriptions factors such as NFκB. To correlate the expression of cell adhesion molecules and NFκB (p65) in leprosy lesions, 30 skin biopsies of patients with leprosy [16 with the tuberculoid (TT) or borderline tuberculoid (BT) forms and 14 with the lepromatous (LL) or borderline lepromatous (BL) forms] were analyzed by immunohistochemistry. A larger mean number of cells expressing VCAM-1 (BT/TT: 18.28 ± 1.4; BL/LL: 10.67 ± 1.2; $p = 0.0002$), ICAM-1 (BT/TT: 9.92 ± 1.1; BL/LL: 5.87 ± 1.0; $p = 0.0084$) and CD62E (BT/TT: 13.0 ± 1.5; BL/LL: 2.58 ± 0.3; $p = 0.0001$) were observed in BT and TT lesions. The mean number of cells expressing NFκB was similar in the two clinical forms (BT/TT: 2.21 ± 2.7; BL/LL: 2.35 ± 3.1;$p = 0.9285$). No significant correlation was observed between expression of the transcription factor and adhesion molecules analyzed. The synthesis of ICAM-1, VCAM-1 and CD62E depends on the activation of NFκB, which acts synergistically with other transcription factors. Adequate activation of intracellular signaling pathways results in the production of endothelial adhesion molecules, contributing to the recruitment of cells to the site of injury and thus eliciting an effective inflammatory response in the elimination of the bacillus.

1. Introduction

Leprosy is a chronic infectious disease caused by the obligate intracellular parasite *Mycobacterium leprae*. The interaction between the bacillus and human immune system determines the clinical manifestations of the disease [1]. These manifestations vary from a localized, resistant form in which a Th1 immune profile predominates, called tuberculoid-tuberculoid (TT), to the opposite form, called lepromatous-lepromatous (LL), characterized by disseminated infection and the predominance of a Th2 profile. Borderline forms [borderline-tuberculoid (BT), borderline-borderline (BB), and borderline-lepromatous (BL)] exist between these two forms whose immunological and clinical characteristics oscillate between the two poles [2].

The recruitment of blood leukocytes to the site of injury is of fundamental importance for the development of an adequate inflammatory response [3]. This process starts with leukocyte rolling in which leukocytes roll on selectins expressed on activated endothelial cells. The step of firm adhesion is mediated by integrins whose ligands are members of the immunoglobulin family, including intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1), which are expressed on the surface of endothelial cells [4]. The firm adhesion of leukocytes to endothelial cells is followed by transmigration [3].

Transcription factor NFκB regulates the expression of inflammatory genes involved in the pathogenesis of inflammatory and infectious diseases, including leukocyte-endothelial adhesion molecules [5]. In the cytoplasm of unstimulated cells, NFκB is bound to an inhibitor, called IκB, which retains it in an inactivated state and prevents its migration to the nucleus. Once stimulated, IκB is degraded, releasing NFκB that can translocate to the nucleus and promote gene transcription [6].

The expression of some leukocyte-endothelial adhesion molecules is

* Corresponding author. Núcleo de Medicina Tropical, UFPA, Av. Generalíssimo Deodoro 92, Umarizal, Belem, Para, 66055-190, Brazil.
E-mail address: juarez@pesquisador.cnpq.br (J.A.S. Quaresma).

https://doi.org/10.1016/j.micpath.2017.11.027
Received 16 September 2017; Received in revised form 11 November 2017; Accepted 18 November 2017
Available online 21 November 2017
0882-4010/

regulated at the site of leukocyte recruitment, for example, E-selectin (CD62E), VCAM-1 and ICAM-1 [4]. The transcription of these proteins can be regulated by exposure to certain inflammatory cytokines such as TNF-α and IL-1β and involves the activation of transcription factors such as NFκB [7].

The objective of the present study was to correlate the expression levels of NFκB (p65) and endothelial adhesion molecules (ICAM-1, VCAM-1 and CD62E) in skin lesions of leprosy correlating with the pattern of immune response already described in the literature. These data may help to better understand the complex host-pathogen interactions in leprosy.

2. Materials and methods

Thirty paraffin-embedded skin biopsies were used, including Sixteen 16 BT or TT (grouped in the resistance or TT pole) and 14 BL or LL (grouped in the suscetible or LL pole) according to the histopathology report and the classification of Ridley and Jopling. The patients were seen at the Dermatology Outpatient Clinic of the Tropical Medicine Center of Federal University of Pará and Dermatology Service of the State University of Pará and the samples were processed at the Laboratory of Immunopathology. The project was approved by the Research Ethics Committee of the Tropical Medicine Center/UFPa (Protocol No.212.969).

The samples were fixed in 10% formalin, dehydrated in alcohol, and embedded in paraffin. The paraffin blocks were cut with a microtome into 5-μm thick sections. The sections were mounted on slides and submitted to immunohistochemistry using the streptavidin-biotin peroxidase method as described previously by Quaresma et al. [8]. The following primary antibodies were used: anti-NFκB p65 (Abcam 32536, dilution 1:200), anti-VCAM-1 (Abcam74514, 1:100), anti-ICAM-1 (Abcam2213, 1:100), and anti-CD62E (Abcam49506, 1:100).

The slides were analyzed under a Zeiss microscope (model 456006) using a 40× objective. An endothelial cell showing brown staining was defined as positive. For NFκB, since it exhibits cytoplasmic and nuclear expression, to correlate with the immune activity, only those cells that showed the labeling pattern of the NFκB activated form with nuclear labeling were quantified. Stained cells were counted in five randomly chosen fields and the mean number of cells per field was calculated.

Statistical analysis of the data was performed with the GraphPad Prism 5.0 program. The results are reported quantitatively and were analyzed using the Student t-test and Pearson's correlation test. A level of significance of $p \leq 0.05$ was adopted.

3. Results

In the TT forms, histopathological analysis of the lesions revealed well-delimited tuberculoid granulomas consisting of histiocytes, epithelioid cells associated with a lymphocytic inflammatory infiltrate that generally surrounded the granuloma. In the LL forms, the loose granulomas consisted of histiocytes and plasma cells associated with a lymphocytic inflammatory infiltrate.

The general pattern of immunohistochemistry showed positive areas in inflammatory cells and endothelium for NFκB, and for ICAM-1, VCAM-1, CD62E only in the endothelium. The labeling pattern for NFκB was cytoplasmic or cytoplasmic/nuclear, depending on whether it was in its activated or non-activated form.

Immunohistochemical analysis showed higher expression of the endothelial adhesion molecules in TT lesions compared to LL lesions and this difference was statistically significant (Table 1). The expression of NFκB was similar in the two forms studied.

No significant correlations were observed between the endothelial adhesion molecules and the transcription factor (Table 2). However, there was a predominance of NFκB in the cytoplasm of endothelial cells as shown in Fig. 1.

Table 1
Quantitative analysis of the expression of transcription factor NFκB and of the adhesion molecules VCAM-1, ICAM-1 and CD62E in the polar forms of leprosy.

Markers	TT (mean ± *SD*)	LL (mean ± *SD*)	*p*
NFκB	2.21 ± 2.7	2.35 ± 3.1	0.9285
VCAM-1	18.28 ± 1.4	10.67 ± 1.2	0.0002*
ICAM-1	9.92 ± 1.1	5.87 ± 1.0	0.0084*
CD62E	13.00 ± 1.5	2.58 ± 0.3	0.0001*

TT – Tuberculous leprosy; LL – Lepromatous leprosy; SD - Standard deviation.
*T Student test ($p \leq 0.05$).

Table 2
Correlation ofNFκBwith endothelial markers in the polar forms of leprosy.

Correlation	TT	LL
NFκB x VCAM-1	$r = 0.1753$ $p = 0.5161$	$r = -0.4928$ $p = 0.0734$
NFκB x ICAM-1	$r = 0.05993$ $p = 0.8255$	$r = -0.03496$ $p = 0.9056$
NFκB x CD62E	$r = 0.1154$ $p = 0.6705$	$r = -0.2620$ $p = 0.3655$

TT – Tuberculous leprosy; LL – Lepromatous leprosy.
*Pearson test ($p \leq 0.05$).

4. Discussion

In the present study, higher expression of the endothelial adhesion molecules (ICAM-1, VCAM-1 and CD62E) was observed in TT leprosy lesions (Table 1). The increase in immunoglobulins and E-selectin at the TT forms of the disease might be associated with the development of an inflammatory response and the recruitment of cells to the site of injury, regulating the pattern of the predominant cytokines.

VCAM-1 is an immunoglobulin that mainly binds to integrin VLA-4. The expression of VCAM-1 is low in the inactivated endothelium, but is rapidly induced after stimulation by IL-1β, TNF-α or lipopolysaccharide (LPS) [9]. Studies indicate that TNF-α is more efficient in increasing VCAM-1 levels than IL-1β [10].

The role of VCAM-1 and of its ligand VLA-4 has been studied in infections with *Leishmania donovani* [11]. The interaction between VCAM-1 and VLA-4 is not directly involved in the recruitment of leukocytes to hepatic lesions; however, blockade of these molecules results in a reduction in the production of IL-12p40 by splenic dendritic cells, an event that contributes to the persistence of infection in the liver. Thus, VCAM-1/VLA-4 participates in the activation of dendritic cells in infections with *L. donovani*. In contrast, in the development of encephalitis caused by *Toxoplasma gondii*, the blockade of the VLA-4 ligand leads to a reduction in the recruitment of T cells in the brain of infected guinea pigs [12]. Additionally, the production of IFN-γ is compromised [12]. In this case, adhesion molecules do not only contribute to the formation of the inflammatory infiltrate but also to the development of an effector immune response against *T. gondii*.

Taken together, the data demonstrate that VCAM-1 plays a role in the pathogenesis of infectious diseases, acting on the recruitment of inflammatory cells and the activation of the inflammatory response, and is associated with the production of cytokines. The pattern of distribution of VCAM-1 in the TT and LL lesions of leprosy studied here suggests that this adhesion molecule influences polarization of the disease.

ICAMs are structurally related to members of the immunoglobulin superfamily and can bind to β2 integrins present on leukocytes. ICAM-1 is expressed constitutively at low levels on endothelial cells. Stimulation by IL-12, TNF-α, IFN-γ or LPS increases the expression of this molecule [13]. In addition to endothelial cells, ICAM-1 is also expressed on other types of cells such as epithelial cells, lymphocytes, monocytes, eosinophils, keratinocytes, and dendritic cells. A biological