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The role of classical and alternative macrophages in the immunopathogenesis of herpes simplex virus-induced inflammation in a mouse model

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ABSTRACT

Background: The exact mechanism of the inflammatory changes occurring during the development of Behçet's disease (BD) remains unclear.
Objective: We investigated the role of classical (M1) and alternative (M2) activation of macrophages in a herpes simplex virus (HSV)-induced BD mouse model.
Methods: The classical *vs.* alternative activated macrophage ratio (M1/M2 ratio) was calculated by analyzing the surface markers CD16/32 and CD23 as M1 and M2 markers, respectively, by flow cytometry. mRNA expression of interferon (IFN)-γ and interleukin (IL)-6 as M1 and arginase-1, FIZZ-1, and MHC-II as M2 markers were analyzed by reverse transcription-polymerase chain reaction. Cytokine levels were assessed by enzyme-linked immunosorbent assay.
Results: The M1 phenotype was upregulated in BD mice, and an increased M1/M2 ratio was observed compared to that in asymptomatic BD normal and normal healthy mice. Recombinant (r)IFN-γ significantly increased the M1/M2 ratio (1.74 ± 0.42) compared with that of rIL-4 (0.83 ± 0.20). BD mice treated with rIL-4 showed a decreased M1/M2 ratio (1.2 ± 0.3) compared to that of the rIFN-γ- (2.1 ± 2.3) treated group and also showed ameliorated BD symptoms accompanied by downregulation of IL-17 and IL-6 and up-regulation of IL-4.
Conclusion: Therefore, modulation of macrophage phenotypes could be an effective therapeutic approach for treating BD in the future.

1. Introduction

Macrophages are highly plastic and can significantly influence T cell responses depending on the environmental signals (*e.g.*, cytokines and chemokines) that they predominantly produce [1]. Macrophages are phenotypically heterogeneous and engage in diverse activities in parallel with the Th1 and Th2 types of adaptive immune responses, which have been termed type 1 (M1) and type 2 (M2) macrophages [2,3].

M1 macrophages are also called classically activated macrophages; they develop in response to endogenous or exogenous inflammatory stimuli such as the Th1 cytokine interferon-gamma (IFN-γ) or lipopolysaccharide (LPS) [4]. M2 macrophages are also known as alternatively activated macrophages. They are regulated by Th2 cytokines such as interleukin (IL)-4 or IL-13 [3]. IL-4 up-regulates a distinctive set of genes, establishing quite a different functional microenvironment than that of M1 macrophages [3].

Behçet's disease (BD) is a chronic, relapsing, inflammatory disorder clinically characterized by bipolar aphthosis and microvascular skin and ocular lesions [5]. The exact etiology of BD remains unclear, but viral infection has long been postulated as one of the main factors. Since Hulusi Behçet first proposed an unclassified viral etiology [6], many researchers have verified this viral hypothesis by detecting herpes simplex virus (HSV) DNA or RNA in the saliva, mononuclear cells, and genital ulcers of patients

Abbreviations: HSV, Herpes simplex virus; BD, Behçet's disease; LN, lymph nodes; SP, spleen; PBMCs, peripheral blood mononuclear cells; TNF, tumor necrosis factor; IL, interleukin; IFN, interferon; LPS, lipopolysaccharide; RT-PCR, reverse transcription-polymerase chain reaction; FACS, fluorescence-assisted cell sorting; TEM, transmission electron microscopy; CD, cluster of differentiation; MHC, major histocompatibility complex; FIZZ-1, found in inflammatory zone 1.

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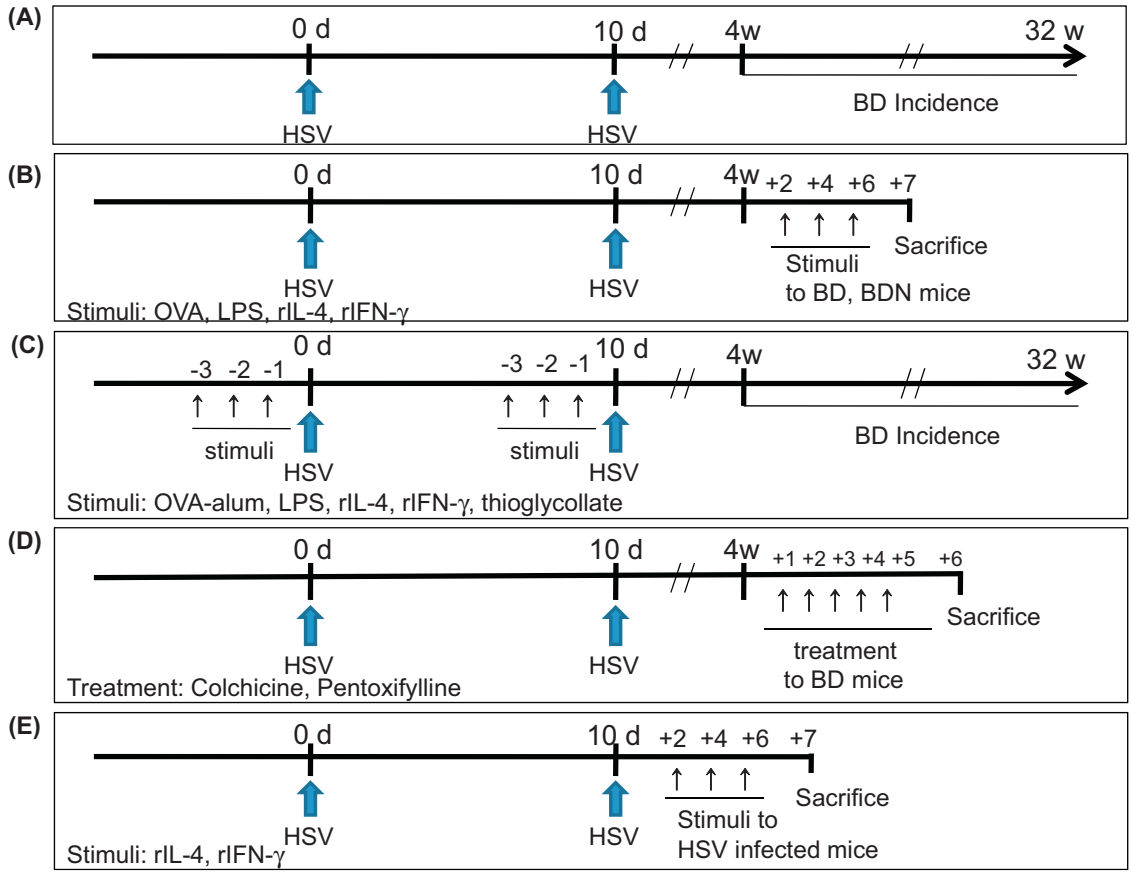

Fig. 1. Schematic diagrams of injection schedule.

with BD [7,8]. Subsequent to these findings, Sohn et al. reported that inoculating the earlobes of ICR mice with HSV type 1 (HSV-1) leads to the development of BD-like symptoms as in humans, such as oral, genital, skin, and intestinal ulcers, as well as ocular and nervous symptoms [9].

Although several immunological abnormalities have been demonstrated, the exact mechanisms of the inflammatory changes that occur have yet to be determined. Serum factors can induce classical pro-inflammatory activation of human peripheral blood macrophages *in vitro* in patients with BD disease, suggesting that serum factors might be responsible for the inflammatory changes in patients with BD [10]. In addition, Mege et al. reported that cultured monocytes of patients with BD produce more tumor necrosis factor (TNF)-α, IL-6, and IL-8, either spontaneously or after stimulation with LPS [11].

However, it is essential to identify different macrophage phenotypes, as particular phenotypes are associated with BD pathogenesis and, more importantly, with disease prevention or recovery. To date, no reports are available about the mechanisms involved during M1 activation in the development of BD in HSV-induced BD-like symptoms in mice. In this study, we investigated the shift in macrophage phenotype and the M1/M2 ratio in mice with HSV-induced BD-like symptoms.

2. Materials and methods

2.1. Animals

In this study, 4–11-week-old ICR male mice were used for experiments, and all animals were handled in accordance with a protocol approved by the Institutional Animal Care and Use Committee of the Ajou University School of Medicine, Republic of Korea (Institutional Approval Number: AMC-101).

2.2. Clarification of BD, BDN, and normal mice

The earlobes of 4–5-week-old mice were scratched with a needle and inoculated with 1×10^6 plaque-forming units/ml of HSV-1 (F strain), which had been grown in Vero cells as described previously [9]. The virus was inoculated twice at a 10-day interval, followed by 32 weeks of observation as shown in the diagram of injection schedule (Fig. 1A). Multiple symptoms were observed after inoculating HSV-1 into mice. Among the symptoms in human patients of Behçet's disease, as outlined on the BD Activity Form (www.behcet.ws/pdf/BehcetsDiseaseActivity-Form.pdf), mouth ulcerations, genital ulcerations, erythema, skin pustules, skin ulcerations, joint arthritis, diarrhea, red eye (right, left), reduced vision (right, left), loss of balance, discoloration, and swelling of the face were selected and two or more symptoms in a mouse was considered BD. HSV-1 infected but asymptomatic mice were used as BD normal (BDN), and mice that were not infected with HSV-1 were used as normal mice.

2.3. BD mice severity score

The score for each BD mouse symptom was one, and the sum of the symptoms in each mouse was used to determine BD severity. The severity score of an individual BD mouse varied from two to four. Disappearance of symptoms or a decrease in the lesion size of >20% was classified as an improvement. This BD severity was followed by determining the BD activity index, as outlined on the BD activity form prepared by the International Society for Behçet's Disease (www.Behcet.ws/pdf/BehcetsDiseaseActivityForm.pdf).