Clinical Immunology (2014) 153, 64–72

available at www.sciencedirect.com

Clinical Immunology

www.elsevier.com/locate/yclim

Involvement of mannose-binding lectin in the pathogenesis of Kawasaki disease-like murine vasculitis

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Received 28 January 2014; accepted with revision 28 March 2014
Available online 8 April 2014

KEYWORDS
Kawasaki disease;
Vasculitis;
Mannose-binding lectin;
Animal model

Abstract Kawasaki disease (KD) is a paediatric idiopathic vasculitis. In this study, on the basis of studies using an established animal model for KD, we report that mannose-binding lectin (MBL) is involved in the pathogenesis of the disease. KD-like experimental murine vasculitis was induced by intraperitoneally administering a *Candida albicans* water-soluble extract (CAWS). MBL-A gradually increased in the serum of the model mice treated with CAWS. Deposition of MBL-A and MBL-C was observed in the aortic root, including the coronary arteries, which is a predilection site in experimental vasculitis. Corresponding to the distribution patterns of MBLs, marked deposition of C3/C3-derived peptides was also observed. Regarding the self-reactivity of MBLs, we observed that MBLs interacted with core histones to activate the lectin pathway. These results suggest that some types of pathogens provoke the MBL-dependent complement pathway (lectin pathway) to cause and/or exacerbate KD-like vasculitis.

1. Introduction

Kawasaki disease (KD) is a paediatric idiopathic vasculitis, which can sometimes result in aneurysms of the coronary arteries [1], leading to an increased risk of myocardial infarction [2]. Although its molecular pathogenesis is still

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http://dx.doi.org/10.1016/j.clim.2014.03.019
1521-6616/

unclear, a line of studies has suggested that an uncontrolled immune response to infectious stimuli is involved in the pathogenesis of KD [3].

In particular, many investigators have reported a possible relationship between KD and superantigens [4–6], which are pathogen-derived substances that activate a specific class of T cells and induce massive production of cytokines [7]. For example, Yoshioka et al. reported that Vβ2/Vβ6.5 T cells, which are responsive to *Streptococcus*-derived pyrogenic exotoxin C, are increased in KD patients [8,9]. Although superantigens have been considered one of the most likely causal factors of KD [10], some studies do not support this hypothesis [6,11,12].

Compared with cellular immunity, little is known about the role(s) of innate humoral immunity in the pathogenesis of KD. However, some population genetic studies have reported a possible relationship between KD and genetic polymorphisms of mannose-binding lectin (MBL) [13–16], a circulating pattern recognition receptor protein, which recognises particular carbohydrate structures in microorganisms to activate the complement pathway (lectin pathway) [17]. The MBL genotype affects the plasma levels of MBL. In KD patients younger than 1 year middle/low MBL-expressing genotype increases the risk of coronary artery lesion (CAL) formation, whereas high MBL-expressing genotype increases the risk of CAL in KD patients older than 1 year [14,15]. These findings suggest that CAL susceptibility is not simply a result of a deficiency of the MBL-dependent innate immune system in response to pathogens, implying that the adverse effects of MBL should also be taken into consideration. In fact, MBL has been implicated in a number of diseases, including rheumatic heart disease, rheumatoid arthritis and ischemia–reperfusion injury [18–20]. However, with the exception of ischemia–reperfusion injury, the pathophysiological roles of MBL remain elusive in these diseases, including KD.

Because limited clinical specimens (e.g. blood and urine) are available for the study of KD, animal models are indispensable tools to investigate the molecular mechanisms underlying the disease. *Candida albicans* water-soluble extract (CAWS)-induced murine vasculitis is one of the most established animal models for KD [21], and it shares pathological features with KD [22]. Although the molecular basis underlying the experimental KD-like vasculitis is unclear, understanding its pathological mechanisms is important for elucidating the aetiology of KD.

In the present study, we employed a KD animal model to investigate the involvement of MBL in the pathogenesis of KD-like murine vasculitis. On the basis of the results, we report that the MBL-dependent complement pathway is involved in the pathological processes of KD-like experimental vasculitis.

2. Materials and methods

2.1. Animals

Male DBA/2CrSlc mice (4–5 weeks old) were used throughout this study. Mice were provided by Shimizu Experimental Materials Co., Ltd. and used in the experimental animal facility of the Kyoto Prefectural University of Medicine. The animal experiments were conducted in accordance with the university's guidelines for experimental animal studies. CAWS was prepared as described in [23]. The murine vasculitis model was prepared by the method established by Ohno and Nagi-Miura et al. [21,23]. In brief, mice were intraperitoneally administered CAWS [1 mg/(mouse·day)] or PBS (sham control group) for 5 days. Mice were then euthanised under general anaesthesia on day 1, 6 or 11 after CAWS administration for 5 consecutive days.

2.2. Pathological study

Four mice per experimental group were used. After washing the blood out by perfusion with PBS(−), the organs were excised and immediately frozen in OTC compound without fixation. The specimens were sectioned using a cryostat to generate 8-μm sections. Immunohistochemical detection was conducted according to the manufacturer's instructions. For the detection of MBL-C, sections were fixed with acetone for 10 min. After incubation in 0.3% hydrogen peroxide/methanol for 20 min, the sections were washed with TBS and then blocked with 10% goat serum-PBS (Nichirei, Tokyo) for 15 min. The sections were incubated with an anti-MBL-C rat monoclonal antibody (1:25 dilution) (HyCult, Uden) in TBS/0.02% Tween20 [TBS-T] containing 3% BSA and 1 mM $CaCl_2$ at room temperature (RT) for 2 h. After

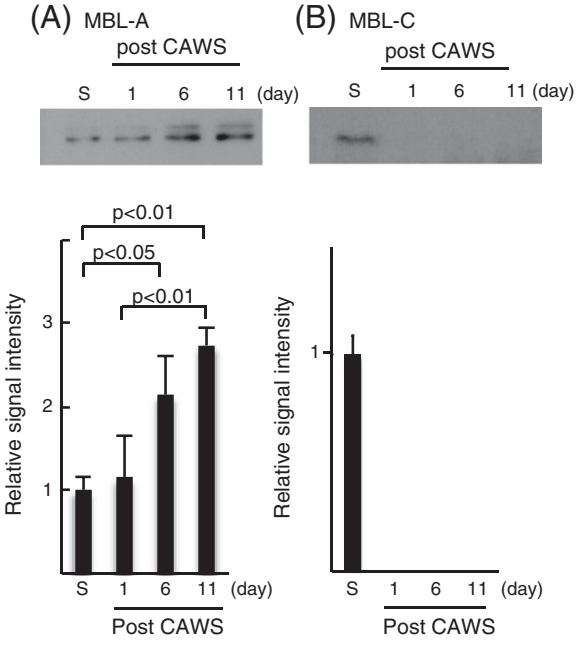

Figure 1 *Candida albicans* water-soluble extract (CAWS)-induced quantitative changes in serum mannose-binding lectin (MBL)-A and -C levels. Time-dependent quantitative changes of (A) MBL-A and (B) MBL-C in the serum of CAWS-treated mice were determined using western blotting. Electrophoresis for MBL-A was performed under non-reducing conditions. The signal intensity of each group was quantified and is shown as mean ± standard deviation (n = 3/group). 'S' stands for serum from the sham control mice. Signal intensities are shown as relative values compared with the sham control. Representative immunoblots are shown at the top of the figure.