Jpn. J. Infect. Dis., 57, 2004

Murine Model of Kawasaki Disease Induced by Mannoprotein-β-Glucan Complex, CAWS, Obtained from *Candida albicans*

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SUMMARY: Intraperitoneal administration of CAWS (water-soluble extracellular polysaccharide fraction obtained from the culture supernatant of *Candida albicans*) to mice induces coronary arteritis similar to Kawasaki disease. We analyzed differences in the production of cytokines involved in the occurrence of coronary arteritis among mouse strains, C3H/HeN, C57BL/6, DBA/2 and CBA/J. The incidence of arteritis was 100% in C57BL/6, C3H/HeN and DBA/2 mice, but only 10% in CBA/J mice. The coronary arteritis observed in DBA/2 mice was the most serious, with several mice expiring during the observation period. The CAWS-sensitive strains revealed increased levels of IL-6 and IFN-γ during the course of a specific response to CAWS by spleen cells. In contrast, IL-10 levels were observed to increase markedly in CAWS-resistant CBA/J mice, but not the CAWS-sensitive strains. However, TNF-α levels were more elevated only in DBA/2 mice. The difference in disease development and cytokine production strongly suggests that the genetic background of the immune response to CAWS contributes to the occurrence of coronary arteritis.

Candida albicans is a clinically important fungus and is known to cause disseminated candidiasis and candidemia in immunocompromised hosts. Analyses have long been conducted on the coagulation reaction of limulus blood cell components with microbial cell components, and the presence of the factor C initiated cascade that reacts with bacterial endotoxins and the factor G initiated cascade that reacts with β-1,3-glucans is known. The factor G cascade is being used for the diagnosis of mycotic contamination and mycotic infections. As was previously mentioned, although patients with deep mycoses have been clearly demonstrated to release β-glucans into the blood, these are present in extremely small amounts, and the overall structure of the factor G activating substance present in the blood is unknown. Although the factor G activating substance has the potential to exhibit various biological activities, this is also unknown. The metabolism of the active components released into the blood from a local site of infection is also unknown. In order to clarify these matters, we conducted research using *C. albicans*.

Biochemical properties of CAWS (1,2): We first cultured *C. albicans* in a completely synthetic medium in order to obtain water-soluble limulus factor G activating substance that is released from the cells, and obtained a water-soluble polysaccharide fraction released into the culture supernatant (*C. albicans* water-soluble fraction: CAWS), which is thought to be similar to the β-1,3-D-glucan actually present in patient blood. CAWS demonstrated a positive reaction to the G-test as expected. It reacted to the G-test as low as 100 ng/mL. The yield of CAWS was approximately 80 mg/L: the polysaccharide content was 70%, the protein content was 10%, the primary component sugars were mannose and glucose (M/G ratio = 6.3 ± 1.3 from *C. albicans* IFO 1385 derived CAWS), and CAWS also reacted with factor serum to the cell wall mannan. According to the results of NMR analysis, CAWS was surmised to have a mannoprotein and a β-1,6-glucan portion, which are the main components of *C. albicans* cell wall. Moreover, fractionation using concanavalin A agarose resulted in separation into column-bound and pass-through fractions, with the column-bound fraction also exhibiting reactivity to the G-test. On the basis of these findings, CAWS was strongly suggested to be a compound that contains mannoprotein, β-1,6-glucan and β-1,3-glucan.

Arteritis induced by CAWS and predicted mechanism (3-6): Kawasaki disease (KD) is a pediatric disease accompanied by acute fever, and its underlying cause remains unknown to date. This disease results in occasionally fatal sequelae such as the formation of aneurysms in the coronary arteries. Although the current standard treatment regimen consists of administration of large doses of globulin preparations, this approach is not always satisfactory. Murata et al. conducted an analysis on children with KD and found that *C. albicans* extract (CADS) isolated from the stool specimens of the patients induced coronary arteritis in mice that resembled KD. During the course of joint research, we found that administration of CAWS according to the standard protocol induced a similar coronary arteritis in mice. Moreover, when additional experiments were conducted on different strains, the resulting coronary arteritis was more pronounced in C3H, DBA/2 and C57B1 mice, and less pronounced in CBA/j mice. Although these differences among strains were similar to the differences among strains observed with CADS by Murata et al., the sensitivity of the DBA/2 mice was different. Moreover, more than half of the DBA/2 mice died during the observation period, suggesting the possibility of a strong manifestation of heart disease (Fig. 1). On the basis of these findings, CAWS-induced coronary arteritis is considered to be a good model for the pathology of arteritis as well as the development of treatment methods.

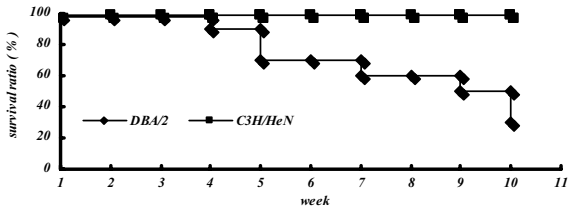

Fig. 1. Survival ratio of CAWS administered mice.
CAWS was administered to DBA/2 and C3H/HeN strains of mice and survival was monitored for 10 weeks.

We therefore attempted to determine the immunological mechanism underlying the arteritis. In strains in which arteritis occurred prominently, splenomegaly occurred frequently and the numbers of neutrophils and macrophages increased. In addition, when spleen cells were prepared immediately after administration of CAWS and cultured in vitro, myeloperoxidase was observed to be released into the supernatant even in the absence of stimulation. In addition, MPO-ANCA levels in the blood were also elevated. On the basis of these findings, neutrophils present in the spleen were suggested to be maintained in an activated state. In addition, spleen cells were re-stimulated with CAWS and cytokine production was compared. The production of cytokines such as IL-6 and IFN-γ was higher in strains in which arteritis was induced. On the other hand, IL-10 production was higher in strain CBA/j that exhibited a low level of induction of arteritis.

Reactivity of DBA/2 mice to fungal glycans (7,8): As mentioned above, DBA/2 is the most sensitive strain to CAWS-induced arteritis, not only from the view point of histology, but also survival. I feel it very close to sudden death of KD-patients carrying aneurysms in the coronary arteries. DBA/2 is a widely used inbred strain that is valuable in a wide number of research areas including cardiovascular

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biology, neurobiology, and sensorineural research, and is known to show a low susceptibility to developing atherosclerotic aortic lesions following 14 weeks on an atherogenic diet. It is of note that the mechanism of CAWS-induced arteritis might not be related to those of atherosclerosis. Thus we planned to analyze the reactivity of DBA/2 mice to fungal glycans and found that DBA/2 contained anti-β-glucan antibody in sera without any active immunization with fungi. In addition, spleen cells of DBA/2 mice strongly reacted with fungal β-glucans to release various cytokines such as IFN-γ, TNF-α, GM-CSF, and IL-12, and the key cytokine was GM-CSF. Characterization of DBA/2 mice for fungal glycan reactivity is still going to concrete the unique property.

CONCLUSIONS

We have discussed the structure and activity of CAWS. Although this research initially started out by focusing on its significance as a means for diagnosing deep mycoses in animal models, since CAWS exhibits various activities in human and mouse, it is clearly a component that provides several extremely interesting topics for future research, such as shock model, endogenous septicemia model and arteritis model. These models are valuable for use as animal models for the treatment of refractory diseases.

CAWS is a compound consisting of mannoprotein, β-1,6-glucan, and β-1,3-glucan portions. In the body, it is metabolized after expressing its activity by means of multiple receptors, such as mannose receptor, mannan-binding protein, complement components, complement receptors, and dectin-1. We previously reported that there are no enzymes in the body that selectively metabolize β-glucans, and that β-glucans are microbial cell components that tend to accumulate in the body. Thus, their basic kinetics in the body differs from that of cellular components having a decomposition system, such as chitin and peptidoglycans. CAWS was found to be mainly deposited in liver. Further analysis must be conducted to determine what types of receptors are used and how CAWS is eliminated from the body.

Study of CAWS is still on the first stage. It is hoped that CAWS will be able to contribute to the elucidation of KD and related diseases, and to develop new therapeutic strategies.

ACKNOWLEDGMENTS

This is summary of joint study of School of Pharmacy, Tokyo University of Pharmacy and Life Science, Department of Bioactive Molecules, National Institute of Infectious Diseases, and Department of Pathology, Ohashi Hospital, Toho University School of Medicine. The author sincerely appreciates to all the staffs and students in our laboratory for technical assistance and to all collaborators for experiments and discussion. This study was supported by a grant for private Universities provided by Ministry of Education, Culture, Sports, Science and Technology, by the Ministry of Health, Labour and Welfare of Japan, and by the Promotion and Mutual Aid Corporation for Private Schools of Japan.

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