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THERAPY WITH IMMUOGLOBULIN AMELIORATES GIANT CELL MYOCARDITIS IN RATS

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Purpose. We investigated the effects of immuoglobulin(IG) upon experimental giant cell myocarditis in rats with an analysis of the phenotypic characteristic of myocardial dendritic cell(DC) and ribonuclease protein assay(RPA) for myocardial cytokines. Also, we investigated which fragment of IG, $F(ab')_2$ or Fc, was effetive to ameliorate myocarditis. **Methods.** Giant cell myocarditis was induced in rats by immunization of porcine cardiac myosin. Human intact IG(1g/kg/day) or human $F(ab')_2$ were administered intraperitoneally everyday until 21st day. Immunohistochemistry was performed to analyze the immunological behavior of DC and other infliltrating cells. In addition, we performed the RPA to confirm the anti-inflammatory action of IG. **Results.** Intact IG therapy significantly ameliorated experimental giant cell myocarditis macroscopically and microscopically, but $F(ab')_2$ fragments did not ameliorate significantly. Immunohistochemichal analysis showed that intact IG therapy suppressed DC expression both during the early and the fulminant phases. The RPA analysis revealed that intact IG therapy completely suppressed the mRNA expressions of interleukin(IL)-1, IL-6, and interferon-γ. **Conclusions.** The present study provides the evidence that IG therapy suppressed giant cell myocarditis due to the suppression of DC expression and the anti-inflammatory actions, and that these effects may be mediated via the Fc portion of IG.

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CANDIDA ALBICANS -EXTRACT CAUSING SYSTEMIC VASCULITIS IN MICE AS AN ANIMAL MODEL OF KAWASAKI DISEASE

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We established a systemic vasculitis mice model involving coronary arteries using *C. albicans*-Extract. This model is evaluated as an animal model of Kawasaki disease (KD) since this model has many similar points of KD. Careful study of this model may provide fundamental information that increases our understanding of the pathogenesis, natural history, and appropriate therapy of this illness. Yeast cells of a *C. albicans* strain isolated from the feces of a KD patient were incubated at 37°C for 72 hours. After harvest, the extract was obtained from the yeast cells using boiling water and KOH. *C. albicans*-Extract suspended in PBS was injected intraperitoneally for five consecutive days at 1st and 5th week. At 9th week, mice were killed under anesthesia, and then, histological features of the arteritis in various organs were observed. There was a difference in susceptibility to vasculitis between inbred mice strains. BALB/c, DBA/2 and CBA/J mice were resistant to vasculitis, however, C3H/HeN and C57BL/6 mice were classified as sensitive strains. Coronary arteries were most frequently involved; aorta and other medium-sized arteries in kidney, retroperitoneum, testis etc. were sometimes involved. Vasculitis was defined as a productive inflammation, which shows dense infiltrates of large mononuclear cells and polymorphonuclear cells at the adventitia and/or media of arteries with an association of a few small round cells. Fibrinoid necrosis was rarely seen. Most of mononuclear cells and polymorphonuclear cells were positive for CD11b, and small round cells were positive for CD4, CD8a or CD45RB. ICAM-1 was usually expressed on endothelial cells of arteries. IFN-γ was expressed on small round cells and large mononuclear cells, but IL-12 and IL-4 were not expressed in any cells. An expression of TNF-α and IL-6 were usually demonstrated on large mononuclear cells, small round cells and endothelial cells.

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CONTRIBUTION OF MYELOPEROXIDASE TO CORONARY ARTERY VASCULITIS ASSOCIATED WITH MPO-ANCA PRODUCTION INDUCED BY *CANDIDA ALBICANS* -DERIVED SUBSTANCES

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The role of myeloperoxidase (MPO) for vasculitis formation accompanied with the production of MPO-specific anti-neutrophil cytoplasmic autoantibody (MPO-ANCA) was analyzed using wild and MPO-deficient mice. MPO-ANCA levels in sera of wild type C57BL/6 mice with vasculitis particularly in the coronary arteries induced by injection of (*Candida albicans*-derived substances (CADS) increased. The MPO-ANCA titers in sera were significantly higher in vasculitis-positive mice than in vasculitis-negative mice, indicating that MPO-ANCA was closely associated with vasculitis formation. However, the increase of MPO-ANCA titers observed in sera of wild C57BL/6 mice were strongly suppressed in MPO-deficient C57BL/6 mice, accompanied with prevention of vasculitis formation. These results show that MPO acted as an antigen for MPO-ANCA production by CADS injection and was followed by the vasculitis formation. Vasculitis did develop in a few MPO-deficient mice, though the incidence of vasculitis was much lower in MPO-deficient mice than in C57BL/6 mice. This result suggests that other antigens structurally similar to MPO may also exist.

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ANALYSIS OF COMPONENT OF *CANDIDA ALBICANS* CELL WALL INDUCING SYSTEMIC VASCULITIS IN MICE

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Aim:We have been investigating pathology about experimental systemic vasculitis. The experimental arteritis mimicking that of Kawasaki disease (KD) can be induced by intraperitoneal injection of alkaline extract which is prepared from cell wall of *Candida albicans* isolated from feces of patients with KD. The present study aims to elucidate particular component of alkaline extract which induces arteritis. **Methods**:Alkaline extract (CADS) was prepared by the method which we had already reported. The component of CADS was analyzed by 1H-NMR. To remove the protein from CADS, Fehling's solution was added to its suspension (Fehling fraction). To remove β1,3-glucan from CADS, it was digested with zymolyase. (Zymolyase fraction). Fractions of protein-rich and mannan-rich were separated from CADS by ammonium sulfate precipitation. Fractions of β1,6-glucan-rich and β 1,3-glucan-rich were obtained by the sodium hypochlorite treatment from the residue of alkaline extracted *C. albicans*. CADS and other fractions were suspended in PBS and injected to mice (C3H/HeN, 4 weeks of age, male) intraperitoneally for five consecutive days in the first week and the fifth week (4mg/mouse/day). Mice were sacrificed in the 9th week. Microscopic observation was performed by routine histological technique. **Results**:By 1H-NMR analysis, CADS is suggested to contain a large amount of mannan, and a small amount of β1,6-glucan, protein, and β1,3-glucan. Incidence of coronary arteritis induced by CADS, other fractions of Fehling, Zymolyase, protein-rich, mannan-rich, β1,6-glucan-rich, and β1,3-glucan-rich is 5/10, 1/10, 4/10, 2/10, 4/10, 3/10, and 1/10, respectively. There was no histological difference among each group. **Conclusion**:These results suggest that mannan and β 1,6-glucan have influence on development of coronary arteritis in this model.

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IMMUNE REACTION OF *CANDIDA ALBICANS* SOLUBLE POLYSACCHARIDE FRACTION CAWS DEVELOPING CORONARY ARTERITIS IN MICE

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Potassium hydroxide extract of *Candida albicans* (CADS) isolated from Kawasaki disease (KD) patient has been known to induce coronary arteritis in mice resembles to KD. Incidence of the coronary arteritis is strain dependent: C3H/HeN; susceptible, DBA/2; resistant. In addition, a water-soluble polysaccharide fraction (CAWS) released from *C. albicans* into synthetic medium induced coronary arteritis by i.p. administration and showed lethality resembled to the anaphylactic shock by i.v.. Lethality was strain dependent: ICR, C3H/HeN; susceptible, DBA/2; resistant. CAWS is mainly composed of a complex of mannoprotein and β-glucan and reacts with limulus factor G test, specific for 1,3-β-D-glucan. Half clearance time of CAWS in DBA/2 mice was 19 min after i.v. administration. Lethality was partially inhibited by pretreatment with yeast mannan and salbutamol. Incidence rate of coronary arteritis by CAWS was significantly higher than that of CADS. Then, various activities of CAWS were examined by i.p. administration to clarify underlying mechanisms for the development of coronary arteritis, i) CAWS induced cell infiltration into peritoneal cavity and spleen in both C3H/HeN and DBA/2. Ratio of macrophages and granulocytes in the spleen was significantly increased, ii) IL-6 and IL-10 production of the spleen cells was increased in both strains of mice, iii) INF-γ production of the spleen cells was enhanced only in DBA/2 mice, iv) myeloperoxidase (MPO) activity of the spleen cells were increased in both strains of mice, v) Anti-MPO autoantibody in sera was induced more significantly in DBA/2 mice, vi) IL-6 and MIP-2 activity of PEC were significantly reduced in both strains of mice. These results suggested that CAWS showed inflammatory activity and immuno-toxicity and IFN-γ production might be related to the susceptibility to arteritis.

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ANALYSIS OF LEUKOCYTE FUNCITIONS IN VITRO ELICITED BY MANNOPROTEIN-B-GLUCAN COMPLEX OF *CANDIDA ALBICANS*, AN INDUCER OF MURINE CORONARY ARTERITIS

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Candida albicans is a medically important fungus, which induces a disseminated candidasis and candidemia in immunocompromised host, and releases polysaccharide fraction into the patient's blood. We had recently found that *C. albicans* released water-soluble polysaccharide fraction (CAWS) into the synthetic medium and demonstrated that CAWS was mainly composed of a complex of mannan and β-glucan. In murine system, CAWS showed lethality resembled to the anaphylactic shock by i.v., and induced coronary arteritis resembled to KD by i.p.. In this study, we examined the biological activity of CAWS in in vitro cell culture and the results were summarized as follows: i) CAWS inhibited proliferation of splenocytes induced by a B cell mitogen, lipopolysaccharide (LPS) and a T cell mitogen, Concanavalin A dose dependently. ii) Viability of these splenocytes monitored by propidium iodide staining was significantly reduced, iii) Addition of CAWS to the in vitro culture of cell lines, mastocytoma P-815, monophage RAW264.7 and fibroblast L-929 significantly reduced growth rate of these cells dose dependently, iv) LPS mediated cytokine synthesis of RAW264.7 was significantly inhibited by CAWS. v) CAWS induced platelet aggregation by human platelet rich plasma, and vi) CAWS inhibited the production of thrombomodulin from human umbilical endothelial cells and the activity was synergistic with TNF. From the observations, CAWS strongly inhibited cellular functions of leukocytes in vitro and the property would partly be mediated by direct cytotoxic property of CAWS. Enhanced production of injured cells in vascular endothelium would be related to the local inflammatory response in the coronary artery.