論文の内容の要約

応用動物科学専攻

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論文題目　Immune-complex-mediated pathogenesis during experimental *Leishmania major* infection

（実験的 *Leishmania major* 感染における免疫複合体依存性病態形成）

Leishmaniasis is caused by the protozoan *Leishmania* parasites. There are three types of the disease: cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis, and visceral leishmaniasis which result from the parasite invasion to skin, nasal-oral mucosa, and internal organs, respectively. The most common type of leishmaniasis is CL in which *Leishmania major* is one of the most important responsible species. CL is widely distributed in the tropics and subtropics with an estimated incidence of 0.7-1.2 million new cases per year. The major manifestation of CL is skin lesion mainly on exposed parts of the body. The typical skin lesion is the nodule with ulcer which can leave permanent scar and disfigurement.

Experimental *Leishmania* infection in mice has been widely used for the immunological study on leishmaniasis. It is known that antibody is not effective in protection against intracellular *Leishmania* infection. On the contrary, antibody might be associated with pathogenesis of CL. A previous study demonstrated that B cell deficient J_H mice showed smaller lesions during *L. major* infection. The skin lesion progression was restored after specific IgG reconstitution correlated with the increasing of IL-10 production. It is well known that the deposition of immune complex (IC) formed by antibody-antigen binding can cause type III hypersensitivity which contributes to the pathogenesis of diseases such as glomerulonephritis, systemic lupus erythematosus and rheumatoid arthritis. Hypothetically, in the skin lesion of CL, IC can be formed by antigens provided by *Leishmania* parasites and antibodies supplied by circulating system as well as local production from plasma cells, and induce type III hypersensitivity. Thus, this study aims to investigate the involvement of type III hypersensitivity in the skin lesion of CL and its

contribution to the pathogenesis of CL using experimental *L. major* infection.

In chapter 1, it was investigated whether anti-*Leishmania* antibodies produced during the infection and *Leishmania* antigens can induce type III hypersensitivity in skin. Arthus reaction is a local type III hypersensitivity occurring in the skin. The injection of antigens into the skin of immunized animal develops Arthus reaction which is characterized by immediate edema followed by neutrophil infiltration within several hours. Mast cell degranulation is also thought to be involved in Arthus reaction. Thus Arthus reaction was tested by injection i.d. of *L. major* soluble lysate antigens (SLA) and recombinant peroxiredoxin (rPrx) into the dorsal skin of *L. major*-infected BALB/c mice (*Lm*-i-BALB/c). At 1 hr after the injection edema was developed at the injection sites. Histopathological examination demonstrated massive neutrophil infiltration at 8 hr after the injection. Furthermore, increased degranulated mast cells were observed at the injection sites at 1 hr after the injection. Hence, it was considered that antibodies generated with *L. major* infection mounted Arthus reaction in an antigen-specific manner. Since IgG is the most abundant antibody and was significantly elevated after *L. major* infection, to confirm that IgG antibodies are responsible for this reaction, isolated IgG from *Lm*-i-BALB/c sera and the mouse monoclonal IgG antibody against peroxiredoxin C11C were injected i.d. into the dorsal skin of naive BALB/c mice followed by immediate injection i.v. of SLA. Edema was developed at the injection sites of antibodies at 1 hr after the injection of SLA. Histopathological examination demonstrated increased degranulated mast cells at 1 hr and massive neutrophil infiltration at 8 hr after the injection at the injection site of antibodies. In addition, isolated IgG did not induce passive cutaneous anaphylaxis, while *L. major*-infected BALB/c sera which contained IgE antibodies did. It further indicated that anti-*L. major* IgG antibodies were responsible for the reverse passive Arthus reaction rather than IgE antibodies. Therefore, anti-*L. major* IgG antibodies produced during the infection and *L. major* antigens can induce Arthus reaction. This suggested the potential of type III hypersensitivity in the skin lesion during experimental CL.

Chapter 2 was devoted to investigate the involvement of type III hypersensitivity in the skin lesion of experimental CL. The immunohistochemical colocalization of antibody and C3 is often used to indicate IC deposition in tissue. Besides, characteristic cell responses of neutrophil and mast cell were demonstrated during Arthus reaction induced by anti-*L. major* IgG antibodies and *L. major* antigens. Thus, the involvement of type III hypersensitivity in the skin lesion of

Lm-i-BALB/c was evaluated regarding IC deposition and the characteristic cell responses. The colocalization of IgG and C3 in serial sections of the skin lesion in *Lm*-i-BALB/c was demonstrated. Massive infiltration of neutrophils and degranulated mast cells were observed in the skin lesion of *Lm*-i-BALB/c. These suggested the involvement of type III hypersensitivity. Recombination-activating gene-2-deficient ($RAG2^{-/-}$) mice lack antibody production, thus never develop type III hypersensitivity. To further elucidate the involvement of type III hypersensitivity in the skin lesion during *L. major* infection, reconstitution of anti-*L. major* IgG antibody into *L. major*-infected $RAG2^{-/-}$ mice (*Lm*-i-$RAG2^{-/-}$) was carried out by repeated administration i.v. of isolated IgG from *Lm*-i-BALB/c sera. Anti-*L. major* IgG antibody level was significantly elevated by IgG reconstitution. IC deposition was indicated by the colocalization of IgG and C3 in the skin lesions in IgG reconstituted *Lm*-i-$RAG2^{-/-}$. More degranulated mast cells and neutrophils were observed in the skin lesion of IgG reconstituted *Lm*-i-$RAG2^{-/-}$ than non-reconstituted *Lm*-i-$RAG2^{-/-}$. Thus, type III hypersensitivity was reproduced in the skin lesion of *Lm*-i-$RAG2^{-/-}$ by IgG reconstitution. This further suggested the involvement of type III hypersensitivity in the skin lesion of experimental CL.

In chapter 3, the contribution of type III hypersensitivity to the progression of skin lesion during experimental CL was evaluated by reproducing type III hypersensitivity in the skin lesion using IgG reconstitution into *Lm*-i-$RAG2^{-/-}$. *Lm*-i-$RAG2^{-/-}$ which lack not only functional B cells but also functional T cells, develop nonulcerative skin lesion different from ulcerative skin lesion in *Lm*-i-BALB/c. Thus, reproducing type III hypersensitivity in the skin lesion of *Lm*-i-$RAG2^{-/-}$ can reveal the pathologic changes caused by type III hypersensitivity without the interference of T cell responses. As a result of IgG reconstitution, *Lm*-i-$RAG2^{-/-}$ developed ulcerative skin lesion similar to *Lm*-i-BALB/c. Furthermore, IgG reconstituted *Lm*-i-$RAG2^{-/-}$ showed larger nodule and more parasite load than non-reconstituted *Lm*-i-$RAG2^{-/-}$. Pathological examination showed the epidermal disruption as well as focal necrosis of the dermis in IgG reconstituted *Lm*-i-$RAG2^{-/-}$ similar to those observed in *Lm*-i-BALB/c. In the dermis of nodule, vasculitis was often observed in IgG reconstituted *Lm*-i-$RAG2^{-/-}$ consisting with *Lm*-i-BALB/c. Vasculitis refers to the histopathological blood vessel damage, primarily caused by neutrophil infiltration. The severity of vasculitis was evaluated by counting the number of small blood vessel with vasculitis. Consistent with *Lm*-i-BALB/c, IgG reconstituted *Lm*-i-$RAG2^{-/-}$ showed a higher number of small blood vessel

with vasculitis than non-reconstituted *Lm*-i-RAG2$^{-/-}$. Properly, ulceration was induced by the dysfunction of blood vessel caused by vasculitis. Thus, the exacerbation of skin lesion by reproducing type III hypersensitivity in the skin lesion of *Lm*-i-RAG2$^{-/-}$ suggested that type III hypersensitivity contributes to the skin lesion progression in experimental CL.

In this study, firstly in chapter 1 it was demonstrated that anti-*L. major* IgG antibodies produced during the infection and *L. major* antigens can induce type III hypersensitivity in skin. In chapter 2, the occurrence of type III hypersensitivity in the skin lesion was suggested by localization of IC deposition and the characteristic cell responses of type III hypersensitivity in *Lm*-i-BALB/c. Furthermore, reproducing type III hypersensitivity in the skin lesion by IgG reconstitution into *Lm*-i-RAG2$^{-/-}$ showed similar IC deposition and cell responses to *Lm*-i-BALB/c. In chapter 3, the contribution of type III hypersensitivity to the progression of skin lesion was revealed by reproducing type III hypersensitivity in the skin lesion using IgG reconstitution into *Lm*-i-RAG2$^{-/-}$ ending up with exacerbated skin lesion, especially the restored ulceration. In conclusion, for the first time, this study described that type III hypersensitivity occurs in the skin lesion of experimental CL and exacerbates the progression of skin lesion. It could help understanding the pathogenesis of CL and yield insights into the development of new diagnostic method and possible immunotherapy of the disease.