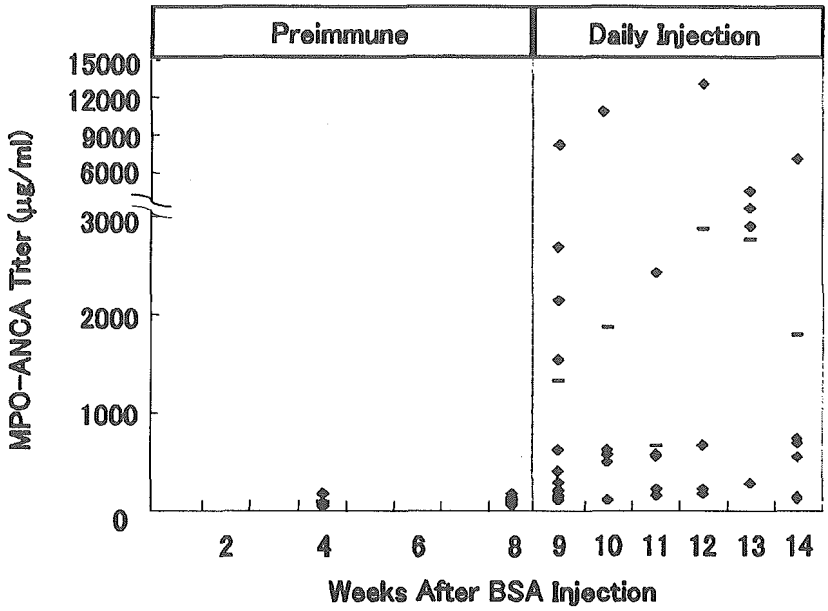
A



B

MPO-ANCA Titer (μg/ml)

	Weeks after BSA Injection	
	4	9 – 14
Group of BSA injected	31 ± 40	112 – 13137 (range)
Control group (Adjuvant only)	1.36 ± 0.10	1.75 ± 0.75[a]

Mean ± S.D.
N=10 except for group of BSA (9–14 weeks)
[a]: data at 14 weeks

C

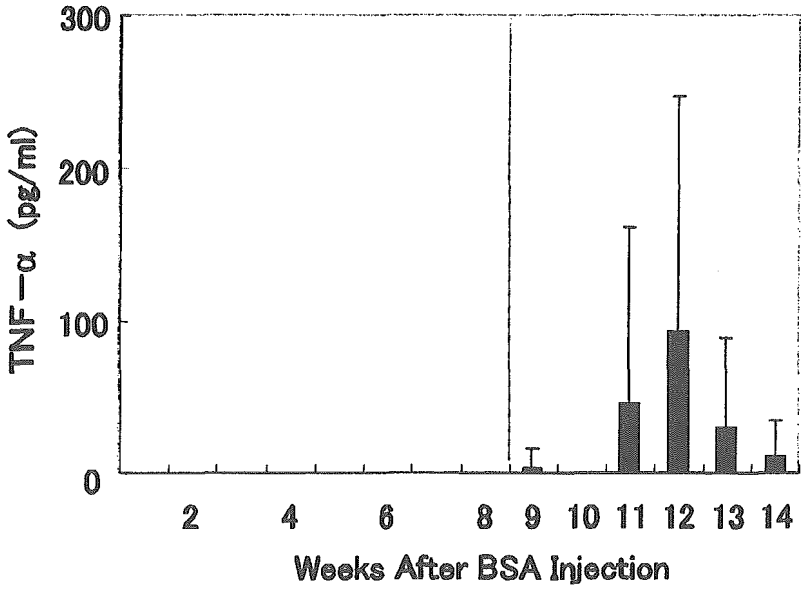


Fig. 3. Increase of MPO-ANCA levels and TNF-α after BSA injection. (A) MPO-ANCA titer, (B) average or range of MPO-ANCA titer, and (C) TNF-α concentration. These were measured with ELISA, as described in "Materials and Methods."

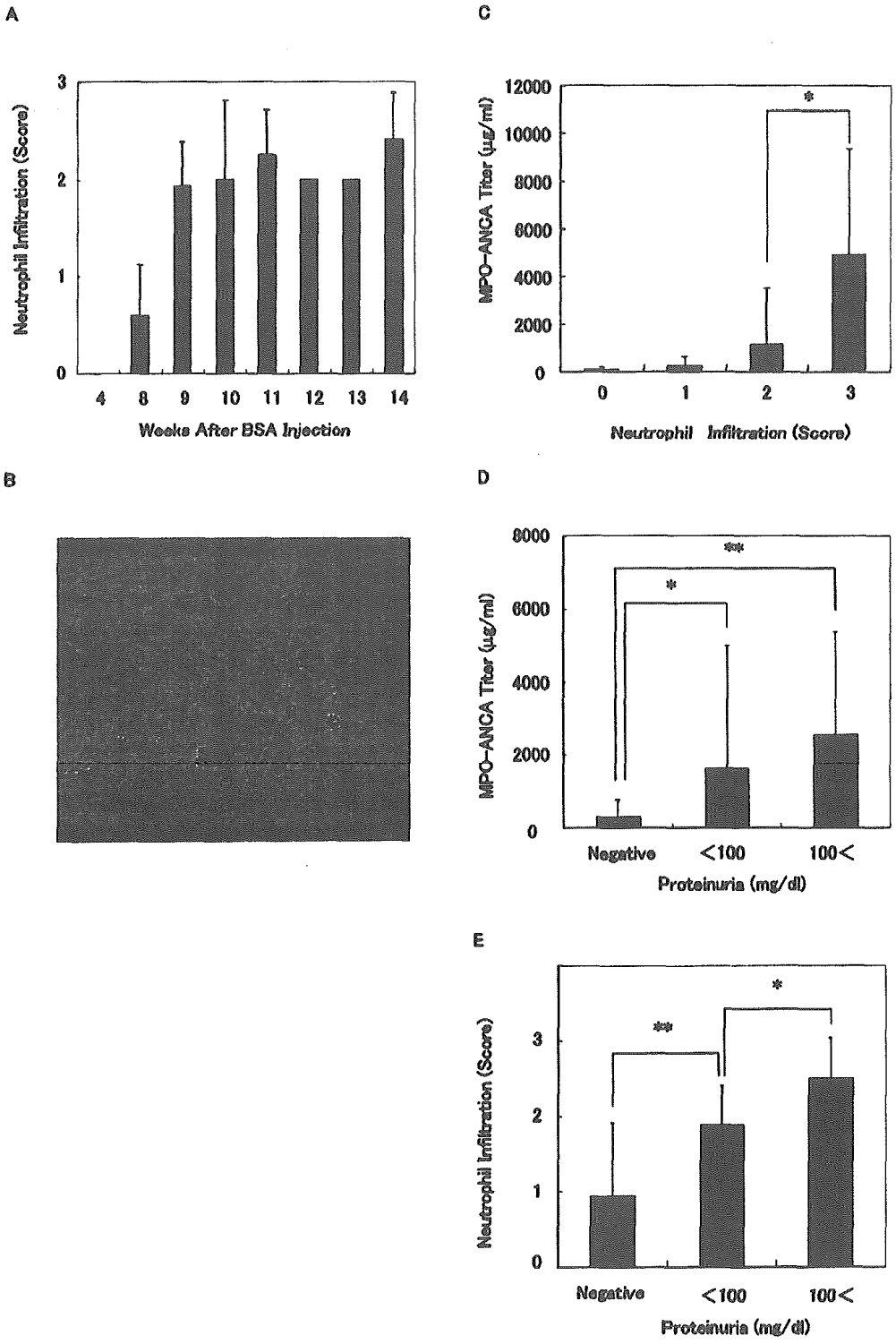


Fig. 4. Neutrophil infiltration into glomerulus and correlations among MPO-ANCA titer, neutrophil infiltration, and proteinuria. (A) Neutrophil infiltration into the glomerulus was observed at 8 weeks and each week from 9 to 14 after the initial BSA injection (N=10). Score of neutrophil infiltration into glomeruli: 0, not detected; 1, mild, less than 3 neutrophils; 2, moderate, 3–5 neutrophils; and 3, marked, more than 15 neutrophils. (B) Neutrophil infiltration into glomerulus stained with anti-mMPO antibody at 14 week. (C) The correlation between MPO-ANCA titer and neutrophil infiltration score: Mice were classified into 4 groups according to neutrophil infiltration: score 0 (N=16), 1 (N=8), 2 (N=35) and 3 (N=6). (D) MPO-ANCA titer and proteinuria (3 groups: negative (N=30), <100 (N=26), 100< (N=9)). (E) Correlation between neutrophil infiltration score and proteinuria. Mean±S.D., *P<0.05, **P<0.01.

been shown (11). We were interested in knowing whether the development of CrGN induced by BSA administration is related to an increase in MPO-ANCA. In the present study, we demonstrated that MPO-ANCA elevation led to the development of CrGN. In human vasculitis, tissue damage occurs not only in the kidney but also in the lung, liver, and other organs, causing organ failure. However, the etiology and relationship between the various patterns of organ involvement have not been well characterized. In MPA with rapidly progressive GN, the disease duration prior to renal failure is not known. In rapidly progressive GN, a kidney biopsy is often carried out late in the course of the disease, when renal failure has already occurred (18). Thus, an experimental animal model is needed to analyze pathogenesis and aid in the development of new treatments. Certain conclusions have already been drawn from existing models, such as the SCG/Kj (6) and Rag2 knockout mice (19).

Our study of GN induced by BSA led us to consider the relationship between lung and kidney damage in experimental vasculitis. After BSA injection, histological features of lung damage appeared before glomerular changes. We also noted marked increases in peripheral leukocyte counts in these mice. These findings indicate that increased neutrophil and platelet counts, positive MPO-ANCA, and activated neutrophils in the glomeruli with proteinuria and hematuria are associated with the development of crescentic GN after serial BSA injections.

Neutrophils and platelets are the dominant inflammatory cells at sites of injury in the initial phase of various immunologically mediated diseases, including the Arthus and Shwarzman reactions and platelet-mediated neutrophil-dependent immune complex nephritis in the rat (10). These studies demonstrate a role for platelets in mediating acute neutrophil-induced glomerular injury and proteinuria. In the present study, the MPO-ANCA titer was slightly elevated at 4 weeks after initial BSA administration and was followed by slight infiltration of neutrophils into glomeruli, with higher MPO-ANCA titer promptly at 9 weeks, indicating renal damage and leading to the development of crescentic formation from proliferative GN. The increase of neutrophils, shown in the present study in peripheral blood in GN induced with BSA, may initiate glomerular injury due to the MPO-H_2O_2-halide system derived by one of the major cytotoxic mechanisms of the neutrophils (14). When the renal artery of rats is perfused with the neutrophil enzyme MPO and its substrates H_2O_2 and chloride, severe endothelial cell injury in the glomeruli and proteinuria was induced, associated with marked activation of platelets and neutrophils (16). Furthermore, rapidly progressive GN in the context of MPO-ANCA vasculitis in humans is usually associated with an increase in the neutrophil count in peripheral blood (16). After this increase, neutrophils release lysosomal enzymes, mainly MPO, into the peripheral blood, then the MPO antibody may be produced to circulate into the peripheral blood. Therefore, increased neutrophil counts with proteinuria may play an essential role in glomerular injury. Kobayashi et al. (15) have reported that injection of rabbit anti-rat MPO antibodies into the rat clearly aggravates nephrotoxic serum nephritis. The administration of anti-MPO antibody induces both glomerular infiltration of activated neutrophils and elevation of proteinuria.

In the present study, we observed a high titer of MPO-ANCA before CrGN after the initial injection of BSA, but this was not crossreactive to the BSA antibody, despite an increase in this antibody. In the initial phase of BSA administration, before 8 weeks, an increase of the anti-BSA antibody may produce an immune complex, resulting in the activation of neutrophils to release lysosomal enzymes, mainly MPO, into the circulating blood. The MPO antibody is then produced to circulate into the blood from 4 weeks after the initial administration of BSA. MPO-ANCA-related GN may develop with the local release of cationic lysosomal enzymes, such as MPO, from invaded neutrophils, which are related to increased peripheral neutrophil and platelet counts. Indeed, MPO itself has been reported to cause CrGN with proteinuria (1). These renal lesions with neutrophil infiltration into glomeruli were followed by an increase in MPO-ANCA in the present study, strongly suggesting that MPO-ANCA production leads to CrGN.

An increase in MPO-ANCA may then result in additional proteinuria and hematuria (9). SCG/Kj mice with the spontaneous formation of CrGN and hematuria have been shown to have glomerular localization of immune complex (IC) (13). In our study, the immune deposition in CrGN was observed in glomeruli after BSA injection. In human ANCA-associated GN, immunofluorescence results usually show pauciimmune GN but partially diffuse granular glomerular immune deposits, suggesting IC deposition (18). Moreover, in ANCA-associated CrGN, a substantial percentage of patients show IC deposition in their renal biopsies (18). IC may therefore trigger vasculitis lesion (17). MPO-ANCA appears to induce CrGN (19) as well other autoantibodies, such as anti-DNA, which was not found in these sera in the present study.

MPO-ANCA induces activation of neutrophils to release reactive oxygen and lysosomal agents, resulting in the development of CrGN through damage of

endothelial cells. Vasculitis and vascular damage in MPO-ANCA-associated GN seem to be caused predominantly by neutrophil activation when these cells adhere to endothelial cells.

Our study demonstrates conclusively that this model mouse is useful for the development of new drugs for the treatment of CrGN and the analysis of mechanisms causing CrGN with vasculitis.

We thank Dr. David Jayne, Addenbrooke's Hospital, Cambridge, for valuable discussions. This study was in part supported by grants from the Ministry of Health, Labour and Welfare, Japan.

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Jpn. J. Infect. Dis., 58, 2005

Modeling on Social Spread from Immunity

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SUMMARY: We are now planning to make a transmission model of infectious diseases in the scale of a city. People live in the city contacting other persons with daily life. The model regards a contact as a source of infection. A person will be simulated as a simple system of differential equations. As a candidate of differential equations, we are now investigating Marchuk's simple model. We adopt Marchuk's simple model because it has formation time, i.e., latent time. As Dr. Takeuchi showed, latent time is very important. There remain problems of choosing parameters for special diseases. We are now planning to use Marquardt method to minimize residuals form clinical data to estimate parameters. As for contacts, there are many approaches. The approach of the MIDAS project is very intensive. Our approach is simple. There are about 30,000 Japanese every fifteen minutes daily life data, sleeping, eating, work, study, house keeping, etc. Our approach is to make virtual families, husband, wife, children in a city and assign actions from the every fifteen minutes data statistically and estimate their contacts in the companies or schools, etc.

We simulated the spread of infectious disease based on the contact model of Japanese people. We also simulated an immune response of a person to get the parameters for the model of contacts of people. When a cold or influenza prevails in winter, schools are shut down in Japan. We investigated the effect of this strategy by simulations of our model.

Immune response

We simulated an immune response of a person as a system of differential equations using Marchuk's simple model (1) shown below. We adopt Marchuk's simple model, because it has formation time, i.e., latent time, one of key factor of infection.

$$\frac{d}{dt}\begin{pmatrix} V \\ C \\ F \\ m \end{pmatrix} = \begin{pmatrix} (\beta - \gamma F)V \\ \xi(m)\alpha V(t-\tau)F(t-\tau) - \mu_c(C - C^*) \\ \rho C - (\mu_f + \eta\gamma V)F \\ \sigma V - \mu_m m \end{pmatrix}$$

where V(t): concentration of pathogenic organ, F(t): concentration of antibodies, C(t): concentration of plasma cells, m(t): relative characteristic of affected organ, β: multiplication, γ: neutralized, τ: formation time, α: antigen-antibody collision *: constant level, μ: life time, ρ: production, η: efficiency, ξ: function of m.

We set 3 days as the parameter of latent time after our experience. The remains of parameters are decided referring a study of pneumonia (2). We used Runge-Kutta method to simulate this delayed differential equations. Period of infection is also an important parameter to make a model. We decided the parameter by the simulation of an immune response. We set 7 days as the period of infection based on the simulations and this coincides with our experiences.

Contact model

People live their lives contacting other persons and infectious diseases spread by contacts. As for infection by contacts, there are many approaches (3-6). The MIDAS project employed a very intensive agent technology to model contacts (7). We used a statistics of Japanese daily life. The statistics is about every 15 minutes actions of daily life of 30,000 Japanese, such as sleeping, eating, work, study, house keeping, etc. (8). Our approach is to make virtual families, i.e., husbands, wives, children in a city, assign their behavior by Monte Carlo method according to the statistics and estimate their contacts in the companies or schools, etc.

Simulation

We assumed 1,000 persons living in a small city and infection spread from a specified family consists of three persons. As for infection, latent time is 3 days and people get infected for 7days and recovered. Infection is not severe, so infected people do not change their behaviors. Infection rate of simulation is assumed as 1%, 10%, 20% and 50%.

In the case of 1% infection prevailed for the longest and the total number of infected people is not so small compared

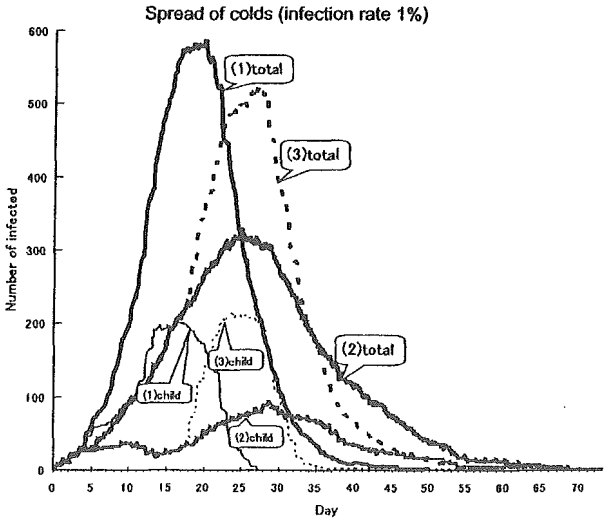

Fig. 1. Number of infected persons in case of infection rate is 1%. (1) Original case, (2) Schools were shut down after 4 days, (3) Schools were shut down after 4 days and opened after 13 days from shutdown. Total numbers of case of (1), (2), (3) are 216,408, 190,488, 217,128 and total numbers of case of (1) child, (2) child (3) child are 63,528, 52,968, 63,528.

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to other cases.

When schools were shut down after 4 days from the outbreak, the peak of infected is decreased but the period of infection is prolonged.

As for children, in case of infection rate is 1%, the total number decreased 17% compared to the original case.

We also simulated the case that schools are opened again after 13 days from school shutdown in infection rate 1%. As for children, the total number does not decrease compared to the original case. The peak of the graph does not decrease also, it only shifted later. This is shown in Fig. 1, graph (1) means original case, graph (2) means the case that schools are shut down after 4 days from outbreak, graph (3) means the case that schools are shut down after 4 days from outbreak and opened again after 13 days from school shutdown.

According to our simulations, strategy of (3) did not improve the situation and the results of (2) showed that the effect of school shut down is not so big.

Our model is simple, but validation is not done well. Professor Koopman cautioned simple models as follows (9). Do not ignore detail and realistic aspect of data. But we can do many parameter runs not only in moderate case but also in extreme cases because of simplicity of the model. Robustness will be gained comparing the results.

ACKNOWLEDGMENTS

This study was partly supported by a grant of Japan Science and Technology Agency.

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Jpn. J. Infect. Dis., 58, 2005

International Symposium on Infectious Agent Transmission Model Building – Focusing on Assessment of Risk to Communities

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SUMMARY: The Susceptible Infected and Recovery (SIR) Model proposed by Robert May in the UK is the basis of the present mathematical model building of infectious disease epidemics. Need for model building incorporating more social and other relevant factors has been recognized. An important example is the introduction of idea of the scale-free distribution of links among the people. More refined models by taking into account the nature of a pathogen, geo-sociological factors, lifestyles of the people, etc., have been developed. For example, Koopman proposed a model for prediction of epidemic expansion based on actual epidemiological data. Eubank proposed a model assessing the bio-terror attack using a model city where every day activity is going on. The present workshop, participated by experts from the US, the UK and Japan, is the first meeting of the proposed series of conference on this issue.

Why mathematical model?

Outbreak of infectious diseases is mostly unpredictable. Continued surveillance and early detection of emerging epidemic are basics of its prevention. Nations have to be prepared for any emergent epidemics. For this, it is essential to know possible consequence of coming plagues. Epidemiological studies of the past cases are useful. However, such studies have limitations. For example, we have experienced no bio-terror attacks in the modern cities. We cannot make assessment by experiments of bio-terrorism for obvious reasons. A mathematical modeling is an alternative approach. Recent progress in computer science has made such an approach more realistic.

Mathematical models developed so far

The Susceptible Infected and Recovery (SIR) model proposed by Robert May (1) is now generally supported and used as a prototype of the mathematical models (Fig. 1). The model is based on the assumption of direct transmission of a pathogen from man to man. However, more parameters, such as geo-sociological elements, lifestyles of different people, climate, transportation system, water supply, etc., have to be incorporated. Several models incorporating such diverse factors have been developed.

Koopman proposed an approach that could be more realistic by incorporating medical and epidemiological data (2). More recently, Eubank (3) proposed a model assessing the bio-terror attack in a model city by using parameters of geo-demography of the city, people's household, people's twenty-four-hour activities, etc.

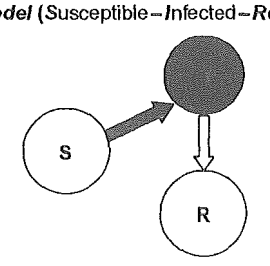

Fig. 1. Traditional modeling

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Present meeting

With the above background, the present symposium was held at the National Institute of Infectious Diseases (NIID), Japan in February 2005 aiming at the review of the ongoing activities in the related field and discussion on the future perspectives (Table 1).

Eubank, Virginia Bioinformatics Institute and Modeling Infectious Disease Agent Study (MIDAS), USA, presented a model of propagation of small pox in a city. Koopman, University of Michigan, USA, proposed an influenza virus spread model, which incorporated the parameters, such as social structures and vaccination options. Yasuda, Josai University, Japan, and Suzuki, NIID, presented a simulation of spread

Table 1. International symposium on trends in transmission models for infectious diseases - 2005: modeling biology focusing to social risk assessment

Program

Opening remarks: Kazuo Suzuki, National Institute of Infectious Diseases, Japan

1. Models for a Science of Infection Transmission
   James S. Koopman, University of Michigan, USA
2. Mathematical Models of the Evolution and Spread of Infections
   Angela McLean, Zoology Department, Oxford University, UK
3. Network Based Models of Infectious Disease Spread
   Stephen Eubank, Virginia Bioinformatics Institute and MIDAS, USA
4. Modeling on Social Spread from Immunity
   Hidenori Yasuda, Josai University, Japan
5. Sensing and Network
   Mami Furukubo, Hitachi Software Engineering, Japan
6. Effectiveness of Vaccination Strategies for Infectious Diseases According to Human Contact Networks
   Fumihiko Takeuchi, Juntendo University, Japan
7. Social Interaction Models
   Takashi Iba, Keio University, Japan
8. Simulation of Human Network
   Kenji Yamamoto, International Medical Center of Japan, Japan

Closing remarks: Takeshi Kurata, National Institute of Infectious Diseases, Japan

Commentators: Hiroshi Yoshikura, National Institute of Infectious Diseases, Japan, and others

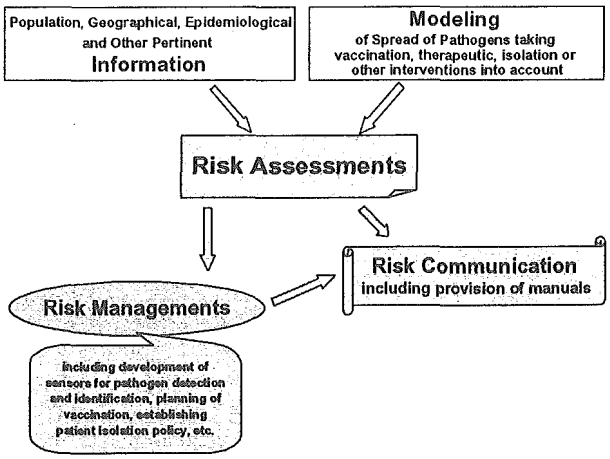

Fig. 2. Transmission models for risk assessment.

of influenza virus incorporating the parameters of immune state of the population and examined the effect of classroom closure on the persistence and spread in a community. Takeuchi presented a mathematical analysis of propagation in a community where man-to-man linking number follows a scale-free distribution. Yamamoto, International Medical Center of Japan, presented a data of hospital infection, frequency of which could be predicted by a mathematical model. McLean presented a model of HIV transmission that is under the effect of frequent mutation. Iba, Keio University, Japan, presented a social interaction model, which could strongly affect man to man spread of pathogens. Kokubo presented a recently developed a pathogen sensor device coupled with reporting and data collection for sensing outbreaks. Commentators were Kurata, Director-General of NIID and Yoshikura, Emeritus member of NIID. The latter commentator discussed influence of social link structure among the homosexuals on the exponential spread of HIV in Japan. Based on discussion in this meeting, reduction of risks of infectious diseases by transmission modeling for infectious agent will be proposed (Fig. 2).

ACKNOWLEDGMENTS

This study was partly supported by a grant of Japan Science and Technology Agency.

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Involvement of Tumor Necrosis Factor-α in the Development of T Cell–Dependent Aortitis in Interleukin-1 Receptor Antagonist–Deficient Mice

Taizo Matsuki, PhD; Kikuo Isoda, MD, PhD; Reiko Horai, PhD; Akiko Nakajima, MSc; Yoshifusa Aizawa, MD, PhD; Kazuo Suzuki, PhD; Fumitaka Ohsuzu, MD, PhD; Yoichiro Iwakura, DSc

Background—Interleukin-1 receptor antagonist–deficient (IL-1Ra$^{-/-}$) mice on the BALB/c background spontaneously develop inflammatory arthropathy that resembles rheumatoid arthritis in humans. These mice also frequently develop aortitis at the root of the aorta, but the mechanism underlying the development of this disease has not been completely elucidated.

Methods and Results—Using IL-1Ra$^{-/-}$ mice (backcrossed 8 generations to the BALB/c background) and wild-type mice, we studied the histopathology and examined the immunologic mechanisms involved in the development of aortic inflammation by cell transplantation experiments. Half of the IL-1Ra$^{-/-}$ mice developed aortitis at the root of the aorta, with massive infiltration of macrophages and monocytes and loss of elastic lamellae in the aortic media. Left ventricular hypertrophy and mild aortic stenosis were also shown by transthoracic echocardiography. Transplantation of T cells from IL-1Ra$^{-/-}$ mice induced aortitis in recipient nu/nu mice. Bone marrow cell transplants from IL-1Ra$^{-/-}$ mice also induced aortitis in irradiated wild-type recipient mice. Furthermore, tumor necrosis factor (TNF)-α deficiency completely suppressed the development of aortitis in IL-1Ra$^{-/-}$ mice, whereas IL-6 deficiency did not affect pathology.

Conclusions—These observations suggest that IL-1Ra deficiency in T cells activates them excessively, resulting in the development of aortitis in IL-1Ra$^{-/-}$ mice in a TNF-α–dependent manner. (***Circulation.* 2005;112:1323-1331.**)

Key Words: interleukins ■ inflammation ■ transplantation

Interleukin (IL)-1 is a major mediator of inflammation and plays important roles in host defense mechanisms through regulation of not only the immune system but also the neuronal and endocrine systems, which interface with the immune system.[1,2] IL-1 consists of 2 molecular species, IL-1α and IL-1β, both of which exert similar but not completely overlapping biological functions through the IL-1 type I receptor (IL-1RI). Another IL-1R, the type II receptor (IL-1RII), has also been identified, but it is not involved in signal transduction; rather, it plays a regulatory role as a decoy. The IL-1R antagonist (IL-1Ra), another member of the *IL-1* gene family, binds to IL-1Rs without exerting agonistic activity. IL-1Ra, IL-1RII, and the secreted forms of IL-1RI and IL-1RII are thought to be negative regulators of IL-1 signaling, participating in the complex regulation of IL-1 activity. Production of both IL-1 and IL-1Ra is induced by a number of other cytokines, bacterial and viral components, and mechanical stresses in a wide variety of cell types, including monocytes/macrophages, epithelial and endothelial cells, and glial cells.[3]

We previously reported that *IL-1Ra* gene–deficient (IL-1Ra$^{-/-}$) mice on the BALB/c background spontaneously developed chronic inflammatory arthropathy.[4] Histopathological analysis showed marked synovial and periarticular inflammation, with articular erosion caused by invasion of granulation tissues closely resembling rheumatoid arthritis in humans. Moreover, elevated levels of antibodies against IgG, type II collagen, and double-stranded DNA (dsDNA) were detected in the sera of these mice, suggesting the development of autoimmunity. Proinflammatory cytokines such as IL-1β, IL-6, and tumor necrosis factor (TNF)-α were overexpressed in the joints of these animals, indicating a regulatory role for IL-1Ra in the cytokine network. Therefore, it was suggested that IL-1Ra is crucial for homeostasis of the immune system.

Received November 15, 2004; de novo received March 16, 2005; revision received May 25, 2005; accepted June 1, 2005;

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Circulation is available at http://www.circulationaha.org

DOI: 10.1161/CIRCULATIONAHA.105.564658

Classic primary vasculitis syndromes such as Takayasu arteritis and giant-cell (temporal) arteritis involve massive recruitment of lymphocytes and macrophages into the vascular wall, destruction of the medial layer with concurrent fibrosis, and proliferation of smooth muscle cells in the intima, leading to neointima formation.[5] Although a number of potential mechanisms, including microbial infection and autoimmune reactions, have been implicated in the development of inflammatory reactions in the vascular system, the precise mechanism underlying the development of vasculitis remains to be elucidated.

Nicklin et al[6] reported that IL-1Ra$^{-/-}$ mice developed aortic inflammation on the 129/O1a×MF1 background. Arterial inflammation with massive transmural infiltration of neutrophils, macrophages, and CD4$^+$ T cells was found at branch points and flexures of the aorta. IL-1β expression was observed mainly in macrophages that were associated with CD4$^+$ cells deep within the vessel wall, suggesting the involvement of CD4$^+$ cells in enhancing IL-1β production. Although the histological changes in the affected IL-1Ra$^{-/-}$ arteries were described in detail, the mechanism underlying the development of arteritis caused by IL-1Ra deficiency was not completely elucidated.

In this investigation, we examined the possibility that autoimmunity is involved in the development of spontaneous arterial inflammation in our IL-1Ra$^{-/-}$ mice on the BALB/c background by cell transplantation experiments. Furthermore, we investigated the role of the proinflammatory cytokines TNF-α and IL-6 in chronic arterial inflammation by generating cytokine-deficient IL-1Ra$^{-/-}$ mice.

Methods

Animals

IL-1Ra$^{-/-}$ mice were produced as described previously.[7] TNF-$\alpha^{-/-}$ and IL-6$^{-/-}$ mice were kindly provided by Dr K. Sekikawa (National Institute of Agrobiological Sciences, Tsukuba, Japan) and Dr M. Kopf (Swiss Federal Institute of Technology, Zurich, Switzerland), respectively. These mice were backcrossed to BALB/c or C57BL/6 mice for 8 generations and then intercrossed with IL-1Ra$^{-/-}$ mice to generate doubly deficient mice (IL-1Ra$^{-/-}$×TNF-$\alpha^{-/-}$ or IL-1Ra$^{-/-}$×IL-6$^{-/-}$ mice)[8]. BALB/c, C57BL/6, and BALB/c-nu/nu mice were purchased from Japan Clea (Tokyo, Japan). A group of wild-type mice of the same age and sex as the test mice was used as a control in each experiment. Mice were housed under specific pathogen-free conditions in an environmentally controlled clean room at the Center for Experimental Medicine, Institute of Medical Science, University of Tokyo. Mice were housed at an ambient temperature of 24°C and a daily light/dark cycle of 12 hours each (light from 8 AM to 8 PM). All experiments were carried out according to institutional ethics guidelines for animal experiments and safety guidelines for gene manipulation experiments.

Histological and Clinical Evaluation for Aortitis and Arthritis

For histological examination of aortitis, mice were anesthetized with pentobarbital and perfused with phosphate-buffered saline (PBS) followed by 10% formalin from an angiocatheter placed in the left ventricule (LV) of the heart. The aorta was fixed in 10% formalin for 48 hours and embedded in paraffin. Serial 10-μm sections of aorta were stained with hematoxylin/eosin for examination of cell infiltration. Masson's trichrome stain was used to evaluate connective tissue damage.[9,10] To detect calcification of the vessel, von Kossa staining, in which sections were treated with 3% $AgNO_3$ and exposed to bright light for 30 minutes, was used. Sections were counterstained with hematoxylin/eosin. Lesion sizes were measured with NIH Image 1.55 software (public domain software). The severity of aortitis was graded on a scale of 0 to 3 by the degree of inflammation near the aortic valve, as follows: grade 0=normal and no infiltration; grade 1=infiltration and loss of elastic lamellae over less than one third of the media of the aortic sinus; grade 2=loss in one third to two thirds of the aortic sinus; and grade 3=loss over more than two thirds of the aortic sinus (see Figure 1).

The incidence and severity of arthritis were judged macroscopically and histologically, as previously described.[4] In brief, each joint was examined weekly for swelling and redness, and severity was graded from 0 to 3 for each paw: grade 0=no special changes; grade 1-light swelling of the joint and/or redness of the foot pad; grade 2=obvious swelling of the joint; and grade 3=fixation of the joint. Severity score was calculated for the 4 legs for a total of 12 points for each mouse. For histological examination, joints were fixed with 10% phosphate-buffered formalin, decalcified in 10% EDTA-4Na, and embedded in paraffin. Sections (4 μm) were stained with hematoxylin/eosin.

Echocardiography

To examine valve function, transthoracic echocardiography was performed with a Sonos 5500 unit (Phillips Co) equipped with 12-MHz and 15-MHz imaging transducers. Mice (female, 40 weeks old) were anesthetized with 2,2,2-tribromoethanol (250 mg/g IP), the chest was shaved, and ECG leads were attached to each limb with needle electrodes. Mice were imaged in a shallow left lateral decubitus position; short- and long-axis views of the LV were obtained by slight angulation and rotation of the transducer. Two-dimensional, targeted M-mode studies were generally taken from the short axis (at the level of the largest LV diameter).

Intraventricular septum thickness, end-diastolic LV internal diameter, end-systolic LV internal diameter, and LV posterior wall thickness were measured. Percent fractional shortening was calculated as [(end-diastolic LV internal diameter)−(end-systolic LV internal diameter)/(end-diastolic LV internal diameter×100)].[11]

Color flow Doppler measurements were used to identify areas of increased (aliased) velocities in the outflow tract from angulated parasternal long-axis views, and these were quantified by pulsed- and/or continuous-wave Doppler. Attempts were made to align the ultrasound beam as parallel as possible with the direction of flow and to record the highest velocities.[12] Then the peak pressure gradient through the LV outflow tract was estimated according to the simplified Bernoulli equation.[13]

Blood Pressure and Heart Rate Measurements

To evaluate hemodynamics, blood pressure and heart rate were measured in nonanesthetized mice (female, 12 weeks old) by the tail-cuff method with a Softron BP-98A device (Softron Co) in the morning. Body and heart weights of these mice were also measured. Values were measured at least 3 times per mouse and were averaged for each individual.

Plasma Cytokine Levels

Proinflammatory cytokine levels in the plasma from 8-week-old male IL-1Ra$^{-/-}$ and wild-type mice were measured by ELISA.[14] Hamster anti-mouse IL-1α monoclonal antibody, hamster anti-mouse IL-1β monoclonal antibody, and polyclonal goat anti-mouse TNF-α antibody (all from Genzyme) were used as capture antibodies. Polyclonal rabbit anti-mouse IL-1α, polyclonal rabbit anti-mouse IL-1β, and polyclonal biotinylated goat anti-mouse TNF-α antibodies (all from Genzyme) were used as secondary antibodies. Detection was performed with horseradish peroxidase–conjugated goat anti-rabbit IgG and horseradish peroxidase–streptavidin (Zymed). TMB substrate was purchased from Dako. IL-6 levels were measured with the OptEIASet mouse IL-6 kit (BD Pharmingen). All assays were performed in duplicate.

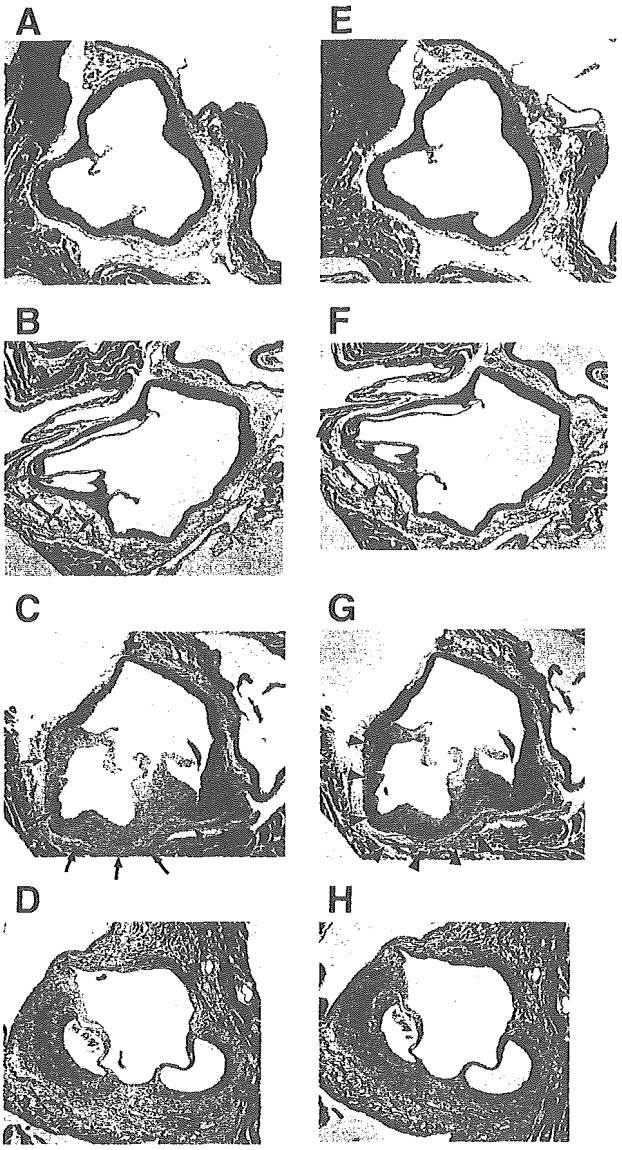

Figure 1. Arterial inflammation around the aortic sinus in IL-1Ra$^{-/-}$ mice. A and E, Normal sections of the aortic valve (score 0) from an 8-week-old, wild-type female mouse. B and F, Mild inflammatory cell infiltration and loss of elastic lamellae over less than one third of the media of the aortic sinus (score 1). Sections from a 4-week-old IL-1Ra$^{-/-}$ male mouse. C and G, Moderate inflammatory cell infiltration and loss of elastic lamellae over one third to two thirds of the media of the aortic sinus (score 2). Sections from an 8-week-old IL-1Ra$^{-/-}$ male mouse. D and H, Severe inflammatory cell infiltration and loss of elastic lamellae over more than two thirds of the media of the aortic sinus (score 3). Sections from an 8-week-old IL-1Ra$^{-/-}$ female mouse. The arrows show inflammatory infiltrates of monocytes and leukocytes, and the arrowheads point to the loss of elastic lamellae. Hematoxylin and eosin stains (A–D) and Masson's trichrome stains (E–H) of the aortic sinus. All images at original magnification of ×40.

T Cell and Bone Marrow (BM) Cell Transplantation

To elucidate the role of T cells in the development of aortitis and arthritis, T-cell transplantation was performed.[12] In brief, cells were prepared from the spleen and lymph nodes of IL-1Ra$^{-/-}$ (n=10, female, 6 to 8 weeks old) and wild-type (n=10, female, 6 to 8 weeks old) mice, and then the cells were treated with hemolysis buffer (17 mmol/L Tris-HCl and 140 mmol/L NH_4Cl, pH 7.2) to remove red blood cells, washed, and passed through a nylon wool column. Then anti-mouse B220 and anti–Mac-1 magnetic bead (Miltenyi Biotec) –treated cells were passed through a MACS column (Miltenyi Biotec) to obtain T cells. The resulting purified T cells were resuspended in 0.2 mL PBS (2×10^7 cells/mouse) and transplanted intravenously into BALB/c-nu/nu mice (n=20, female, 6 weeks old). The development of aortitis in recipient mice was analyzed 10 weeks later.

For BM cell transplantation, BM cells were taken from femurs, tibias, and pelves of IL-1Ra$^{-/-}$ (n=17, female, 5 to 6 weeks old) and wild-type (n=14, female, 5 to 6 weeks old) mice and were treated with hemolysis buffer. T cells were removed by treating the BM cells with anti-mouse Thy1.2 magnetic beads and passing the cells through an MACS column. Purified BM cells (10^7 cells/mouse) in 0.2 mL PBS were transplanted intravenously into lethally irradiated (750 rad) recipient mice at 4 weeks of age (IL-1Ra$^{-/-}$, n=12, female; wild-type mice, n=17, female). The recipient mice were histologically examined 12 and 24 weeks later.

TABLE 1. Incidence of Aortitis in IL-1Ra$^{-/-}$ Mice

Age, wk	Incidence (Rate, %)	Median Score
4	2/5 (40)	1
8	3/6 (50)	2
12	5/10 (50)	2

The number of diseased mice among the total number of animals is shown. The number of male mice studied was 3, 3, and 4 and of female mice was 2, 3, and 6 at 4, 8, and 12 weeks, respectively. Severity of aortitis was graded on a scale of 0–3 by the degree of inflammation of the area near the aortic valve, as detailed in text.

Statistical Analysis

All values were calculated as the mean±SD except where indicated. Fisher's exact test was used for evaluation of the incidence of aortitis between unpaired groups. To compare the values between 2 independent groups, we used the Student *t* test for echocardiographic and hemodynamic values, tissue weights, and cytokine levels. To compare discontinuous values between 2 independent groups, such as aortitis severity score, we used the Mann-Whitney *U* test. A value of $P<0.05$ was considered significant.

Results

Development of Aortitis in IL-1Ra$^{-/-}$ Mice

IL-1Ra$^{-/-}$ mice on the BALB/c background spontaneously developed arterial inflammation beginning at the age of 4 weeks, and ≈50% of them were affected by the age of 12 weeks (Table 1). Interestingly, on the C57BL/6J background, there were no signs of arterial inflammation (data not shown), suggesting the involvement of background genes in the development of aortitis; a similar observation has been made in the case of arthritis.[4] Inflammation developed at several sites in the artery, including the region of the coronary artery ostium near the aorta (Figure 1). Arterial inflammation in IL-1Ra$^{-/-}$ mice was not influenced by sex (incidence of 58% [7/12] in male mice and of 45% [5/11] in female mice at 10 to 14 weeks old; $P=0.42$ by Fisher's exact test). IL-1Ra$^{-/-}$ mice also developed mild myocarditis in the subepidermal pericardium at low incidence (data not shown).

Infiltration of monocytes and occasionally neutrophils was observed in the aorta and valve, and a loss of elastic lamellae in the aortic media was observed on histological examination. Monocytes/macrophages and some neutrophils infiltrated the inflammatory sites in the aortic sinus (Figure 2A). Thus,

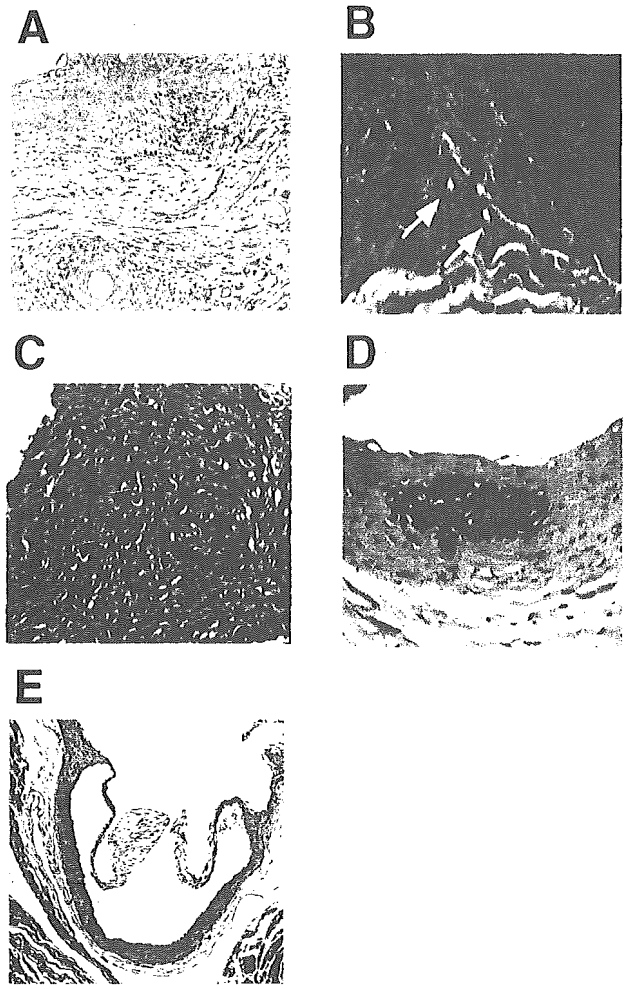

Figure 2. Characterization of arterial inflammation in IL-1Ra$^{-/-}$ mice. A, Inflammatory cell infiltration in the media and adventitia in a 12-week-old IL-1Ra$^{-/-}$ female mouse. B, Formation of microvessels (white arrows) in a section from an 8-week-old IL-1Ra$^{-/-}$ male mouse. C, Chondrocyte-like cells (arrowheads) and calcification (D) in the media in sections from an 8-week-old IL-1Ra$^{-/-}$ female mouse. E, Sections of aortic valve cusp from an 8-week-old IL-1Ra$^{-/-}$ female mouse. Hematoxylin and eosin staining (A, D–E) and Masson's trichrome staining (B and C). Magnification: A and D, ×100; B, ×150; C, ×400; E, ×50.

aortic inflammation may have characteristics of both the acute and chronic phases. We found numerous examples of neovascularization at sites of severe lesions (score 3; Figure 2B). Chondrocyte-like cells were observed in most of the aortas of IL-1Ra$^{-/-}$ mice, although no such cells were observed in the aortas of wild-type mice (Figure 2C). The chondrocyte-like cells were detected at sites of severe inflammation that also exhibited loss of elastic lamellae in the media. To determine whether calcification existed within arterial walls, the sections were stained with hematoxylin/eosin and von Kossa's technique after eosin staining. Calcification of the media of the aorta was observed in ≈30% of affected IL-1Ra$^{-/-}$ mice (Figure 2D and data not shown).

Correlation Between Aortitis and Arthritis

As shown in Table 2, 53% of IL-1Ra$^{-/-}$ mice developed aortitis by 14 weeks of age, whereas 95% of these mice developed arthritis at 14 weeks of age. However, the mutant mice developed aortitis as early as 4 weeks of age, a time when they had not yet developed arthritis (Table 2). Although most of the mice that developed aortitis also developed arthritis, a few of them developed aortitis only without any sign of arthritis at 14 weeks of age (Table 2). This observation was confirmed by histological examination of the joints of IL-1Ra$^{-/-}$ mice that had developed aortitis (data not shown). Thus, the development of aortitis is not necessarily correlated with the development of arthritis.

Development of Cardiac Hypertrophy in IL-1Ra$^{-/-}$ Mice

Because the aortic valve plays a crucial role in heart function and arterial inflammation in IL-1Ra$^{-/-}$ mice occurs specifically in the aortic sinus, we took echocardiograms of IL-1Ra$^{-/-}$ and wild-type mice to examine valve function under conditions of Avertin anesthesia (Table 3). The thickness of both the interventricular septum wall and the LV posterior wall was notably increased. In contrast, LV end-diastolic and end-systolic dimensions and fractional shortening, which are reported to be influenced by Avertin anesthesia,[15] were unchanged, suggesting that the effect of anesthesia was low, if at all. Pressure gradient and flow velocity were significantly increased in IL-1Ra$^{-/-}$ mice. These results suggest that LV function is normal, that the pressure gradient is affected by mild aortic stenosis, and that LV hypertrophy may be induced by pressure overload.

Furthermore, we measured blood pressure and heart rate in 4 IL-1Ra$^{-/-}$ mice and compared these values with these of 4 wild-type mice (Table 4). IL-1Ra$^{-/-}$ mice showed normal blood pressure, but they also showed a small but significant decrease in heart rate under nonanesthetized conditions. The heart weight of IL-1Ra$^{-/-}$ mice was similar to that of wild-type mice, as was their body weight (Table 4).

TABLE 2. Correlation Between Aortitis and Arthritis in IL-1Ra$^{-/-}$ Mice

Aortitis	Arthritis	Incidence at 4 Weeks of Age (Rate, %)	Incidence at 6–8 Weeks of Age (Rate, %)	Incidence at 10–14 Weeks of Age (Rate, %)
–	–	3/5 (60)	0/9 (0)	0/19 (0)
–	+	0/5 (0)	5/9 (56)	9/19 (47.5)
+	–	2/5 (40)	0/9 (0)	1/19 (5)
+	+	0/5 (0)	4/9 (44)	9/19 (47.5)

The number of diseased mice among the total number of animals is shown. Pathological examination of IL-1Ra$^{-/-}$ mice (male, n=3, 8, and 12; female, n=2, 3, and 7) was performed at 4, 6–8, and 10–14 weeks of age, respectively. Data for males and females of the same age were pooled, because no difference between males and females was observed.

TABLE 3. Echocardiographic Measurements in IL-1Ra$^{-/-}$ and Wild-Type Mice

	Wild Type	IL-1Ra$^{-/-}$	P
Interventricular septal wall thickness, mm	0.74±0.11	1.20±0.22*	0.0015
Posterior wall thickness, mm	0.75±0.11	1.14±0.14*	0.0004
End-diastolic diameter, mm	0.19±0.04	0.21±0.04	0.2900
End-systolic diameter, mm	0.080±0.020	0.082±0.025	0.5300
Fractional shortening, %	57.1±3.7	60.6±5.6	0.1367
Flow velocity, cm/s	94±14	181±51*	0.0028
Pressure gradient, mm Hg	3.6±1.1	14.0±7.1*	0.0061

Values are mean±SD. Wild-type mice n=5; IL-1Ra$^{-/-}$ mice n=5 (female, 40 weeks old).
*$P<0.05$ vs wild-type mice (Student t test).

Development of Aortitis in Mice That Received Transplants of IL-1Ra$^{-/-}$ T Cells or BM Cells

We have previously reported that IL-1Ra$^{-/-}$ mice showed increased levels of total IgG, IgG1, or IgE and autoantibodies against Igs, type II collagen, and dsDNA, suggesting involvement of an autoimmune mechanism in the development of disease in this mouse strain.[4] The observation of abundant CD4$^+$ T-cell infiltration at sites of arterial inflammation in IL-1Ra$^{-/-}$ mice also supports this notion.[6] Thus, we examined the role of T cells in the development of aortitis by peripheral T-cell transplantation. Transplantation of T cells from wild-type mice induced mild aortitis at a low incidence in nu/nu mice. In contrast, T cells from IL-1Ra$^{-/-}$ mice induced aortitis at a much higher incidence. The severity score was also significantly increased in this experimental group, indicating that T cells are involved in the development of aortitis in IL-1Ra$^{-/-}$ mice (Figure 3A and 3B and Table 5). To determine whether IL-1Ra deficiency in T cells itself or T-cell sensitization in IL-1Ra$^{-/-}$ mice was important for the development of aortitis, we performed IL-1Ra$^{-/-}$ BM cell transplantation into wild-type recipients. Irradiated control mice without BM cell transplantation died within 2 weeks. Wild-type mice that received wild-type BM cells did not develop any arterial inflammation. A high incidence (100% and 71% at 12 and 24 weeks after transplantation, respectively) of aortitis was observed in wild-type mice that received BM cells from IL-1Ra$^{-/-}$ mice (Figure 3C and 3D and Table 5). When wild-type BM cells were transplanted into IL-1Ra$^{-/-}$ mice, no protective effect on the development of aortitis was observed (incidence of 100% and 33% at 12 and 24 weeks after transplantation, respectively). These results demonstrate that IL-1Ra deficiency in T cells is responsible for the development of aortitis.

TABLE 4. Hemodynamics and Weights in IL-1Ra$^{-/-}$ and Wild-Type Mice

	Wild Type	IL-1Ra$^{-/-}$	P
Heart rate, bpm	554.6±18.3	483.6±18.7*	0.001
Systolic pressure, mm Hg	118.3±14.9	112.5±10.8	0.275
Heart weight, mg	151.7±8.5	134.0±15.0	0.915
Body weight, g	22.9±1.0	22.0±1.7	0.371

Values are mean±SD. Wild-type mice n=4; IL-1Ra$^{-/-}$ mice n=4 (female, 12 weeks old).
*$P<0.05$ vs wild-type mice (Student t test).

Suppression of Aortitis in TNF-α–Deficient but Not IL-6–Deficient, IL-1Ra$^{-/-}$ Mice

It has been suggested that TNF-α and IL-6 are involved in the development of cardiovascular diseases.[16] Therefore, we studied the roles of TNF-α and IL-6 in the development of aortitis in IL-1Ra$^{-/-}$ mice by generating doubly gene-deficient mice. The aortic valves of TNF-α$^{-/-}$–IL-1Ra$^{-/-}$ or IL-6$^{-/-}$–IL-1Ra$^{-/-}$ mice were histologically analyzed at 14 or 8 weeks of age, respectively. Interestingly, TNF-α$^{-/-}$–IL-1Ra$^{-/-}$ mice showed no signs of arterial inflammation, whereas ≈50% of the IL-1Ra$^{-/-}$ mice developed aortitis (Figure 4 and Table 6). On the other hand, the incidence of aortitis was increased in IL-6$^{-/-}$–IL-1Ra$^{-/-}$ mice, although the difference was not statistically significant (by Fisher's exact test, $P=0.09$). The severity score was comparable to that in IL-1Ra$^{-/-}$ mice. These observations indicate that TNF-α is crucial for the development of aortitis in IL-1Ra$^{-/-}$ mice.

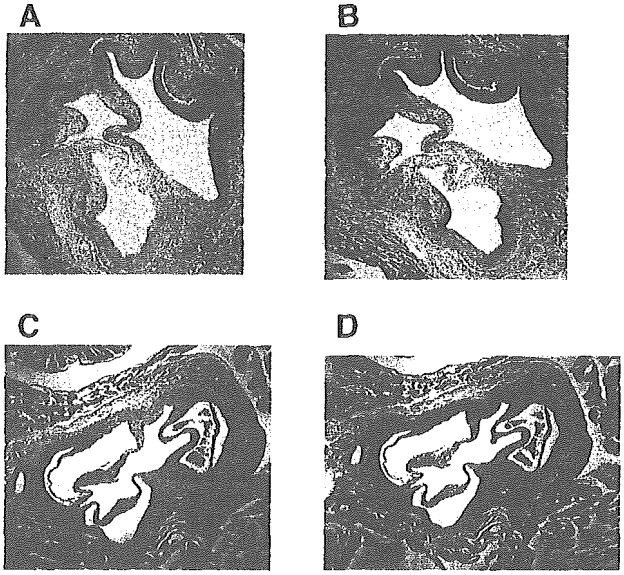

Figure 3. Induction of aortitis by transplantation of IL-1Ra$^{-/-}$ peripheral T cells and BM cells. A and B, Sections from nu/nu female mice 10 weeks after transplantation of T cells from IL-1Ra$^{-/-}$ mice (score 3). C and D, Sections from a wild-type, female mice 12 weeks after transplantation of BM-derived cells from IL-1Ra$^{-/-}$ mice (score 3). Hematoxylin and eosin staining (A and C) or Masson's trichrome staining (B and D). Original magnification ×40.

TABLE 5. Transplantation of T Cells and BM Cells

Donor Mice→Recipient Mice	Incidence (Rate, %)	Median Score
T cell transplantation		
IL-1Ra$^{-/-}$→nu/nu	12/13 (92)*	2†
Wild type→nu/nu	2/6 (33)	1
BM cell transplantation		
12 Weeks later		
IL-1Ra$^{-/-}$→wild type	6/6 (100)	2
Wild type→IL-1Ra$^{-/-}$	6/6 (100)	2
Wild type→wild-type	0/2 (0)	NA
24 Weeks later		
IL-1Ra$^{-/-}$→wild type	5/7 (71)	2
Wild type→IL-1Ra$^{-/-}$	2/6 (33.3)	1
Wild type→wild type	0/2 (0)	NA

NA indicates not applicable. The number of diseased mice among the total number of animals is shown.

*P=0.017, vs wild-type mice by Fisher exact test.

†U value was significant (P<0.05) vs wild-type mice by the Mann-Whitney U test.

In IL-1Ra$^{-/-}$ mice, TNF-α protein levels in the blood were slightly higher than in wild-type mice, whereas the levels of IL-1α, IL-1β, and IL-6 were normal compared with wild-type mice (Table 7).

Discussion

In this report, we have demonstrated that T cells play a crucial role in the pathogenesis of aortitis in IL-1Ra$^{-/-}$ mice on the BALB/c background and that TNF-α is essential for development of the disease. Inflammation of the cardiovascular system was preferentially observed at the aortic root of IL-1Ra$^{-/-}$ mice. As a result, these mice developed mild aortic stenosis and hyperplasia of both the interventricular septum wall and the LV posterior wall. However, the severity of these phenotypes seemed to be much milder on the BALB/c background than on the 129/O1a×MF1 background,[6] in which not only the aortic root but also the main arteries were affected at a high incidence, especially at branch points. It is possible, however, that this apparent difference may reflect not that due to genetic backgrounds but to the ages of the mice, because exact ages of the mice were not known in the preceding report.[6] Shepherd et al[17] also recently reported that IL-1Ra$^{-/-}$ mice on the BALB/c background spontaneously develop aortitis. These authors reported that these mice also spontaneously develop cutaneous inflammation, and we also observed similar signs in our IL-1Ra$^{-/-}$ mice (authors' unpublished observations). Shepherd et al reported that aortic inflammation was normally observed in IL-1Ra$^{-/-}$ (BALB/c×C57BL/6) F_2 hybrid mice as in IL-1Ra$^{-/-}$–BALB/c mice, whereas arthritis was rarely seen in the hybrid mice, suggesting that different background genes are involved in the development of aortitis and arthritis.

At the aortic root of IL-1Ra$^{-/-}$ mice, infiltration of monocytes and macrophages was observed frequently, but accumulation of foam cells, which are derived from macrophages and cause atherosclerosis, was not observed. Occasional infiltration of neutrophils was observed. Loss of elastic lamellae in the aortic media and occasional calcification of the media, signs of degenerative processes that mainly reflect degradation of smooth muscle cells,[18,19] were observed in these mice. Neovascularization was also frequently observed, reflecting inflammation. These pathological findings resemble some aspects of Takayasu arteritis or polyarteritis nodosa in humans, in agreement with a previous report.[6]

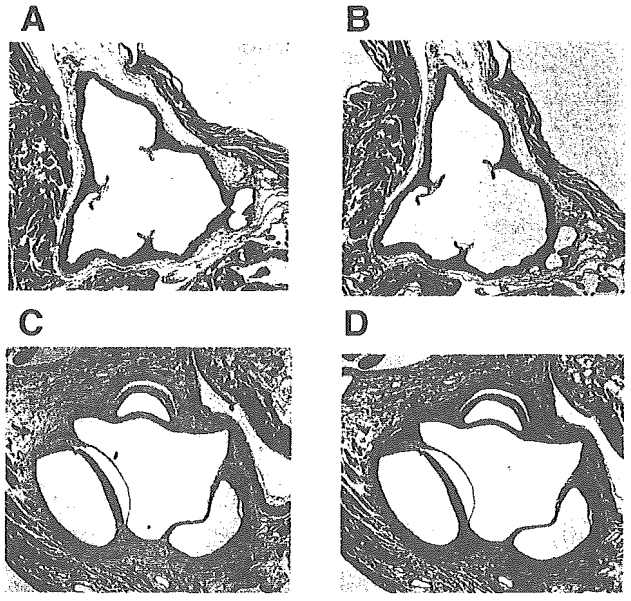

Figure 4. Complete suppression of the development of aortitis in TNF-α–deficient, IL-1Ra$^{-/-}$ mice. Sections of the aortic valves from TNF-$\alpha^{-/-}$–IL-1Ra$^{-/-}$ female mice (14 weeks old, score 0) (A and B) and IL-6$^{-/-}$–IL-1Ra$^{-/-}$ female mice (8 weeks old, score 3) (C and D). Hematoxylin and eosin staining (A and C) or Masson's trichrome staining (B and D). Original magnification ×40.

TABLE 6. Incidence of Aortitis in IL-1Ra$^{-/-}$ Female Mice Crossed With IL-6$^{-/-}$, TNF-$\alpha^{-/-}$ Mice

Group (Age, wk)	Incidence (Rate, %)	Median Score
IL-6$^{+/+}$, IL-1Ra$^{-/-}$ (8)	3/6 (50)	1
IL-6$^{-/-}$, IL-1Ra$^{-/-}$ (8)	6/6 (100)*	3
TNF-$\alpha^{+/+}$, IL-1Ra$^{-/-}$ (14)	5/9 (55)	2
TNF-$\alpha^{-/-}$, IL-1Ra$^{-/-}$ (14)	0/7 (0)†	NA

NA indicates not applicable. The numbers of diseased mice among the total number of animals is shown. Severity scores were not significantly different vs control mice by the Mann-Whitney U test.

*P=0.090, †P=0.028 vs control mice by Fisher exact test.

TABLE 7. Plasma Levels of Proinflammatory Cytokines in IL-1Ra$^{-/-}$ and Wild-Type Mice

Cytokine Level, pg/mL	Wild Type	IL-1Ra$^{-/-}$	P
IL-1α	15.9±7.7	14.9±4.0	0.891
IL-1β	28.9±17.3	47.6±27.6	0.230
TNF-α	111.1±6.2	208.4±18.0*	0.001
IL-6	46.7±35.3	72.5±18.4	0.753

Values are mean±SD. Wild-type mice n=6; IL-1Ra$^{-/-}$ mice n=5 (male, 8 weeks old).

*P<0.05 vs wild-type mice (Student t test).

We have demonstrated that peripheral T cells from IL-1Ra$^{-/-}$ mice can cause aortitis in nu/nu mice, suggesting that activated and/or memory T cells are generated and involved in the development of aortitis. Because IL-1Ra deficiency in BM cells could induce aortitis in wild-type recipient mice, it was suggested that T-cell intrinsic disjunction rather than abnormality of positive-negative selection of T cells in the thymus was responsible for the development of aortitis. With regard to this concept, we have shown that the development of arthritis in IL-1Ra$^{-/-}$ mice was also dependent on T cells.[8] We showed that IL-1 signaling activates T cells by enhancing CD40L and OX40 expression on T cells and causes the development of autoimmunity.[4,20,21] Furthermore, we showed that IL-1Ra is produced by CD4$^+$ T cells and regulates the action of IL-1 in an autocrine manner.[8] Thus, we suggest that IL-1Ra–deficient T cells are excessively activated even by physiological levels of IL-1 and may lose tolerance for aortic endothelial cell components, resulting in the development of autoimmunity and inflammation.

It is known that a small portion of mainstream aortic flow is intercepted during systole by the sinus ridge, or the downstream corner of the sinus of Valsalva; this fluid curls back toward the ventricle to form a large eddy, or vortex, that spins within the sinus cavity and generates turbulence.[22] Hemodynamic force may affect structural and metabolic aspects of vascular endothelial cells,[23] and high shear forces on the leaflet may lead to increased cell damage or turnover,[24] resulting in production of IL-1 from these cells. Indeed, it is known that IL-1 release is increased at the aortic root or at the branch point of the aorta where cells are exposed to mechanical stress caused by blood flow.[25] Therefore, in the absence of IL-1Ra, T cells near the areas where cells are exposed to mechanical stress may be excessively activated.

In IL-1Ra$^{-/-}$ mice, serum levels of myeloperoxidase anti-neutrophil cytoplasmic antibodies, which increase in some types of systemic vasculitis in humans, were not increased (data not shown), although the levels of other autoantibodies such as anti-IgG and anti–type II collagen were increased.[4] These pathologies closely resemble human systemic vasculitis that is typically not associated with anti-neutrophil cytoplasmic antibodies (polyarteritis nodosa, Takayasu arteritis, and giant-cell arteritis). The pathogenic antigens in the aorta in this model remain to be elucidated.

We have shown that most of the mice that developed aortitis also developed arthritis, suggesting that these 2 diseases have a similar pathogenesis (or mechanism). Indeed, we have shown that both diseases are caused by a T cell–dependent mechanism. However, considering the facts that aortitis begins to develop earlier than arthritis and that a large proportion of mice develop only 1 of the diseases, either aortitis (5%) or arthritis (47%), at 14 weeks of age, the pathogenic processes underlying these diseases may be different in part.

Interestingly, we found that TNF-α deficiency suppressed the development of aortitis in IL-1Ra$^{-/-}$ mice. In contrast, IL-6 deficiency in IL-1Ra$^{-/-}$ mice showed pathological findings of aortitis. These results indicate that TNF-α plays an important role in the development of aortitis. TNF-α deficiency but not IL-6 deficiency also suppressed the development of arthritis in IL-1Ra$^{-/-}$ mice.[8] Consistent with these observations, circulating levels of TNF-α but not of IL-6 were increased in IL-1Ra$^{-/-}$ mice. In this context, it is known that activation of antigen-presenting cells by activated T cells through interaction with CD40/CD40L induces TNF-α.[26] Thus, TNF-α production may be enhanced in antigen-presenting cells through interaction with activated T cells in IL-1Ra$^{-/-}$ mice. Furthermore, we previously reported that TNF-α production was induced in T cells by IL-1 stimulation[27] and that T cell–derived TNF-α played an important role in the pathogenesis of contact hypersensitivity[27] and arthritis.[8] TNF-α production by CD4$^+$ T cells is also induced on stimulation with anti-CD3 monoclonal antibody, and IL-1Ra$^{-/-}$ T cells produce significantly higher levels of TNF-α together with IL-4 and interferon-γ than do wild-type T cells in culture supernatants.[8] Other investigators have also reported the production of TNF-α in T cells[28,29] and the presence of TNF receptors in aortic smooth muscle and endothelial cells.[30] Thus, excess TNF-α produced by IL-1Ra$^{-/-}$ T cells and antigen-presenting cells may activate endothelial cells to produce excessive amounts of various inflammatory cytokines and chemokines, resulting in the development of inflammation.[31] It is also known that TNF-α induces the expression of vascular cell adhesion molecule-1 in endothelial cells, which promotes early adhesion of mononuclear leukocytes to the arterial endothelium at sites of inflammation.[32] Although transfer of TNF-α$^{-/-}$–IL-1Ra$^{-/-}$ T cells into nu/nu mice will help evaluate the contribution of T cell–derived TNF-α to the development of aortitis separately from that of antigen-presenting cells, we were unable to address this question because of the difference in the major histocompatibility locus between TNF-α$^{-/-}$ mice (H-2 locus b/b) and BALB/c-nu/nu mice (H-2 locus d/d), even after 8 generations of backcrossing to BALB/c strain.

Taken together, our observations suggest that excessively activated T cells are responsible for the development of aortitis and that TNF-α mediates the inflammatory process. Autoimmune responses against specific antigens on vessel walls may thus be induced, as in the case of arthritis in these mice. However, further analysis is necessary to confirm this finding, because it is also possible that excessively activated T cells directly induce inflammation by producing inflammatory cytokines without the involvement of autoimmunity. Nonetheless, it is possible that both aortitis in IL-1Ra$^{-/-}$ mice and anti-neutrophil cytoplasmic antibody–associated systemic vasculitis in humans share a similar pathogenic process involving TNF-α. Consistent with this notion, it was recently reported that infliximab, an anti–TNF-α antibody, improved endothelial dysfunction in anti-neutrophil cytoplasmic antibody–associated systemic vasculitis in humans.[33] These observations provide new insights into the pathogenesis of vasculitis, and the IL-1Ra$^{-/-}$ mouse should be a useful model to study the pathogenic mechanisms of vasculitis.

Acknowledgments

This work was supported by grants from the Ministry of Education, Science, Sport and Culture of Japan; the Ministry of Health and Welfare of Japan; and the Japan Society for the Promotion of Science. We thank Drs Kenji Sekikawa (National Institute of Animal Health, Japan) and Manfred Kopf (Swiss Federal Institute of Tech-

nology, Zurich) for kindly providing TNF-$\alpha^{-/-}$ mice and IL-$6^{-/-}$ mice, respectively. We also thank Drs Katsuko Sudo and Aya Nambu for technical support and critical comments. We thank all members of our laboratory for their discussions and help in animal care.

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CLINICAL PERSPECTIVE

Vasculitis syndromes such as Takayasu arteritis and giant-cell arteritis involve massive recruitment of lymphocytes and macrophages into the vascular wall, destruction of the medial layer with concurrent fibrosis, and proliferation of smooth muscle cells in the intima, leading to neointima formation. Although a number of potential mechanisms, including microbial infection and autoimmune reactions, have been implicated in the development of inflammatory reactions in the vasculature, the precise mechanism underlying the development of vasculitis remains to be elucidated. In this issue, we showed that IL-1Ra$^{-/-}$ mice, in which excess IL-1 signaling is induced under physiological conditions owing to deficiency of the antagonist, spontaneously develop aortitis at the root of the aorta, with massive infiltration of macrophages and monocytes and loss of elastic lamellae in the aortic media. LV hypertrophy and mild aortic stenosis were also shown by transthoracic echocardiography. These pathological findings resemble some aspects of Takayasu arteritis or polyarteritis nodosa in humans, indicating that IL-1Ra$^{-/-}$ mice are a good model for these vascular diseases. Interestingly, transplantation of T cells from IL-1Ra$^{-/-}$ mice induced aortitis in recipient nu/nu mice, suggesting involvement of T cells in pathogenesis. Furthermore, TNF-α deficiency completely suppressed the development of aortitis in IL-1Ra$^{-/-}$ mice, whereas IL-6 deficiency did not. These observations indicate that both IL-1 and TNF-α play crucial roles in the development of aortitis in IL-1Ra$^{-/-}$ mice. Therefore, control of either IL-1 or TNF-α activity may be beneficial for the treatment of vasculitis in humans.

Original Paper

nephron Clinical Practice

Nephron Clin Pract 2006;102:c35–c42
DOI: 10.1159/000088313

Received: February 11, 2005
Accepted: May 30, 2005
Published online: September 19, 2005

Clinical Efficacy of Intravenous Immunoglobulin for Patients with MPO-ANCA-Associated Rapidly Progressive Glomerulonephritis

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Key Words
Intravenous immunoglobulin · MPO-ANCA · Tumor necrosis factor-α

Abstract
Background: To determine whether intravenous immunoglobulin (IVIg) can control disease activity in patients with myeloperoxidase-antineutrophil cytoplasmic antibody (MPO-ANCA)-associated rapidly progressive glomerulonephritis (RPGN). ***Methods:*** Twelve patients with serologically and histologically confirmed MPO-ANCA-associated RPGN (7 men, 5 women; mean age 71 ± 3 years) received IVIg (400 mg/kg/day) alone for 5 days. The effects of IVIg were evaluated by white blood cell counts, serum C-reactive protein levels, Birmingham Vasculitis Activity Score, rate of change in reciprocal creatinine (1/Cre), and plasma tumor necrosis factor-α levels after IVIg administration. Corticosteroids with or without cyclophosphamide were commenced after IVIg. ***Results:*** After IVIg treatment, a significant decrease was observed in white blood cell count ($p < 0.05$), C-reactive protein values ($p < 0.001$), and Birmingham Vasculitis Activity Score ($p < 0.001$) concomitant with the amelioration of systemic symptoms. The rate of change in 1/Cre significantly improved ($p < 0.05$). Plasma tumor necrosis factor-α levels that were significantly elevated in patients before IVIg compared with normal controls ($p < 0.0001$), rapidly declined after IVIg with a significant reduction ($p < 0.05$). Three months post-treatment with IVIg, all patients showed improvement of disease without serious infectious complications. ***Conclusion:*** IVIg is a potential component of remission induction therapy for patients with MPO-ANCA-associated RPGN.

Introduction

Antineutrophil cytoplasmic antibody (ANCA)-associated rapidly progressive glomerulonephritis (RPGN), which occurs in Wegener's granulomatosis (WG) and microscopic polyangiitis (MPA) [1], leads to renal failure through systemic vasculitis and diffuse crescentic glomerulonephritis. Since crescent formation has features of delayed-type hypersensitivity and is accompanied by the presence of T cells, macrophages, and fibrin in the glomerular lesion [2], high-dose corticosteroids and cyclophosphamide (CYC) are standard treatment for ANCA-associated RPGN; however, such immunosuppressive therapy is often complicated by severe infection in elderly patients [3]. Therefore, to induce early remission of

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the disease including renal insufficiency and to avoid fatal side effects, it is important to establish a therapeutic regimen that can maintain the immune potency of such patients.

Intravenous immunoglobulin (IVIg) has been advocated as a safe and effective treatment for other immune-mediated diseases, such as Kawasaki disease, idiopathic thrombocytopenic purpura, Guillain-Barré syndrome, and chronic inflammatory demyelinating polyneuropathy. European investigators have recently shown that IVIg is clinically useful and safe when administered in conjunction with immunosuppressive drugs, helps suppress disease activity for at least 1 year, and consequently reduces the total dose of immunosuppressive agents in patients with ANCA-associated vasculitis, mainly WG with or without RPGN [4, 5]. Accumulating evidence suggests that it works in multiple phases of immune response; neutralization of circulating pathogenic antibodies, Fc receptor modulation and blockade, or suppression of antibody-dependent cellular toxicity, natural killer cell function, autoantibody production, and complement activation [6]. In addition, Guillain-Barré syndrome patients who received IVIg showed clinical recovery in parallel with reduction in serum levels of tumor necrosis factor-alpha (TNF-α), suggesting an important role of IVIg in inhibiting cytokine activity [7].

Here we report a study evaluating the effectiveness of IVIg as an initial treatment, preceding corticosteroids with or without CYC, in 12 patients with MPO-ANCA-associated RPGN. Since it remains unclear whether IVIg is independently effective [8], we investigated the potential immunomodulatory effect unique to IVIg by comparing renal function, clinical score (Birmingham Vasculitis Activity Score, BVAS), and circulating TNF-α levels before and after IVIg treatment. Since MPO-ANCA-associated RPGN is more common than those related to PR3-ANCA in Asia, which clearly contrasts with the incidence in Western countries, we were able to recruit sufficient numbers of patients with MPO-ANCA-associated RPGN to examine the results statistically. This is the first report of the effects of IVIg in MPO-ANCA-specific RPGN patients.

Patients and Methods

Patients

Twelve consecutive patients with MPO-ANCA-associated RPGN (7 men and 5 women; mean age 72 years; range 57–83 years), who were admitted to the Nephrology Department of Kyoto University Hospital and Kitano Hospital between January 2001 and February 2003, were enrolled in this study (table 1). All patients were diagnosed as having MPA because of elevated serum MPO-ANCA as well as characteristic pathology observed in the renal biopsy specimen before treatment. In all patients, the disease was confirmed based on the definition of MPA described by the Chapel Hill Consensus Conference [9]. Renal involvement was seen in all patients. Patient with rapid aggravation of renal dysfunction with more than 30% rise in serum creatinine (Cre) levels were defined as having RPGN. All except 1 (patient No. 2) were newly diagnosed patients who had been transferred from other hospitals due to the onset of RPGN. Patient 2 had previously demonstrated MPO-ANCA-associated RPGN and recovered after 6 years of treatment with prednisolone and CYC. He again developed fever, arthralgia, and myalgia with elevation of white blood cells (WBC), C-reactive protein (CRP), Cre (33% rise) and MPO-ANCA levels, leading to a diagnosis of MPA recurrence. All patients provided written informed consent for renal biopsy as well as treatment according to the protocol. The hospital Ethical Committee approved the study design.

Histological Evaluation

All renal biopsy specimens showed MPA. Hematoxylin and eosin, periodic acid-Schiff, periodic acid silver-methenamine, Masson trichrome, and elastica van Gieson stain were performed. Direct immunofluorescence studies were performed using frozen sections of renal tissue. Histological activity was assessed as follows: active crescent formation (%) = number of glomeruli with cellular and fibrocellular crescent formation/number of glomeruli without global sclerosis × 100. Each biopsy specimen was scored by two pathologists independently. If there was disagreement between the scores, patients were re-examined by the team to determine a final diagnosis by consensus.

Treatment Protocols

After serum MPO-ANCA, WBC, CRP, renal biopsy, and BVAS were all evaluated to establish a definite diagnosis and activity grading, IVIg was administered intravenously as an initial treatment for 5 consecutive days (400 mg/kg/day). Patients 1–10 received freeze-dried sulfonated human normal immunoglobulin (Kenketsu Venilon-I, Teijin Co., Ltd, Tokyo, Japan), and patients 11 and 12 received freeze-dried polyethylene glycol-treated human normal immunoglobulin (Kenketsu Glovenin-I, Nihon Pharmaceutical Co., Ltd, Tokyo, Japan). Both preparations were free from IgG aggregations that can form in prepared solutions containing sucrose. During IVIg treatment, none of the patients received any other immunosuppressive agents, any blood transfusions, or any intravascular volume repletion treatment. Following the post-IVIg treatment evaluation of clinical scores and laboratory data, all patients received immunosuppressive treatment with oral corticosteroids with or without CYC (table 2). Oral corticosteroids (prednisolone 0–1.0 mg/kg/day) were administered dependent on disease severity and patient age. Methylprednisolone pulse and oral CYC (25–50 mg/day) were also administered to 3 of 12 and 8 of 12 patients, respectively.

Assessment of Disease Activity

Complete blood count and serum markers such as CRP, Cre, and MPO-ANCA were evaluated at the onset of disease, before, and immediately after (mean 6.3 days, range 0–17 days) IVIg, and 1 and 3 months after IVIg. Disease activity was assessed by BVAS before and immediately after IVIg and 1 and 3 months after IVIg. BVAS consists of 59 predefined items derived from clinical, radio-

Table 1. Characteristics of the 12 patients (M/F = 7/5)

Patient	Age	Sex	Data before treatment								
			WBC/μl	CRP mg/l	Cre μmol/l	MPO-ANCA, EU	Active crescents, %	BVAS	extrarenal manifestations	pulmonary involvement	latent and antibiotics resistant infections
1	82	F	7,000	80	283	239	81	19	S, F, E		HBV carrier
2	75	M	9,600	178	106	435	0	23	S, F, A, C, N		MAC, *K. pneumoniae*
3	61	F	8,100	43	126	244	71	15	S, F		
4	82	M	9,400	104	417	159	71	14	S, A		
5	64	F	12,100	154	737	276	81	19	S, F, L	infiltrate	
6	59	M	10,200	139	389	140	90	15	S, F		*Aspergillus*
7	83	F	4,700	1	258	617	60	20	S, F, Ab		
8	82	F	14,700	113	210	306	38	21	S, F, N		
9	57	M	10,700	99	357	980	64	19	S, A, N		
10	62	M	10,100	101	732	370	80	25	S, L, N	nodules	
11	75	M	9,300	60	1,012	82	33	27	S, E, L	infiltrate	HBV carrier, MRSA, *P. aeruginosa*
12	67	M	11,900	68	401	1,740	78	19	S, L	hemoptysis	MRSA
Mean	71		9,820	95	419	466	62	20			
Reference range			3,500–9,100	<3	<106	<20		0			

WBC = White blood cell count; CRP = C-reactive protein; Cre = serum creatinine; MPO-ANCA = myeloperoxidase antineutrophil antibody; BVAS = Birmingham Vasculitis Activity Score; S = systemic symptom (malaise, myalgia, weight loss); F = fever; A = arthralgia; C = cutaneous; E = ear-nose-throat; L = lung; Ab = abdomen; K = kidney; N = neuropathy; HBV = hepatitis B virus; MAC = *Mycobacterium avium* complex; *K. pneumoniae* = *Klebsiella pneumoniae*; MRSA = methicillin-resistant *Staphylococcus aureus; P. aeruginosa* = *Pseudomonas aeruginosa*.

Table 2. Treatment and outcomes after IVIg treatment

Patient	Initial immunosuppressive treatment just after IVIg				Treatment after 3 months			
	mPSL pulse	PSL dosage mg/kg/day	CYC dosage mg/kg/day	dialysis	PSL dosage mg/kg/day	CYC dosage mg/kg/day	dialysis	Cre μmol/l
1	–	0.3	0.7	–	0.3	0	–	124
2	–	0.7	0.4	–	0.3	0.4	–	88
3	–	1.0	1.0	–	0.5	1.1	–	92
4	–	0.5	–	HD	0.7	–	–[1]	308
5	–	1.0	1.0	–	0.6	1.1	–	204
6	1 g, 3 days	0.7	0.8	–	0.3	0	–	177
7	–	0.5	1.3	–	0.3	0	–	203
8	–	0.6	–	–	0.4	–	–	87
9	1 g, 3 days	0.7	0.7	–	0.2	1.7	–	141
10	–	0.8	0.8	–	0.4	0.8	–	353
11	–	0	–	HD	0.5	–	HD	723[2]
12	0.5 g, 3 days	0.5	–	–	0.5	–	–	131
Mean		0.6	0.8[3]		0.4	0.6[3]		173[4]

mPSL pulse = Methylprednisolone pulse therapy; PSL = prednisolone; CYC = cyclophosphamide; Cre = serum creatinine; HD = hemodialysis.

[1] Cessation of HD; [2] Cre level before a hemodialysis; [3] mean CYC dose of 8 patients; [4] patient 11 was excluded.