Bull. Yamanashi Med. Col., 6, 8—14 (1989)

A New Function of Immunoglobulin
—Induction of a Cytokine by IgG Molecule from Autoimmune Mouse—

Kachio TASAKA*, Toshiko SAKIHAMA*, Masahiro IWAMOTO**
Fumito AKASU**, Toshimasa ONAYA**, Yuri SHIRAKURA-SHIBATA***
Shunji MATSUDA****, and Yasuo NAKAJIMA*

The major function of immunoglobulins (Igs) that is widely known is recognition and binding of specific antigens. In addition to this, they have other biological functions such as activation of complement, aggregation of platelets, and degranulation of basophils and mast cells.

A new function of Ig was detected in an autoimmune mouse model, the induction of growth factor (s) from the FDC-P2/185-4 cell line and stimulation of cell proliferation via an autocrine mechanism.

Such a function of immunoglobulins might play an important role in the pathogenesis of autoimmune diseases.

Key Words : IgG, Autoimmune, Autocrine, IL-3

Introduction

The main function of immunoglobulins (Igs) is of course to recognize and bind specific antigen(s). However, immunoglobulins also have other biological functions, such as opsonization, activation of complement, degranulation of basophils and mast cells, aggregation of platelets, and passive immunization of the fetus.

These two different types of functions are carried out by different parts of the immunoglobulin molecule, namely the Fab and Fc portions. The Fab portion plays an immunological role, while the Fc portion fulfils the various biological roles (Fig. 1).

Recently, we detected a new function of immunoglobulin in an autoimmune mouse model, the induction of cell proliferation via the Fc portion.[1),2),3)]

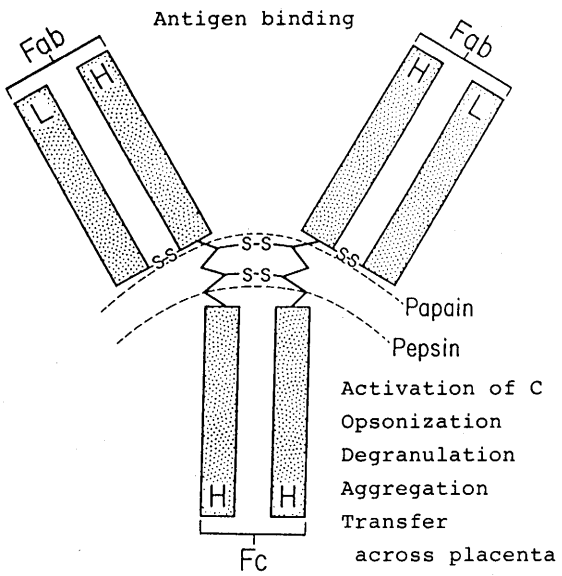

Fig. 1 Modified from the Textbook of Immunology, 2nd ed. by Unanue E.R. & Benacerraf B., Williams & Wilkins.

Yamanashi Medical College, Tamaho, Nakakoma, Yamanashi, 409-38
* Department of Parasitology & Immunology
** Third Department of Internal Medicine
*** Laboratory of Cell Technology
**** Department of Immunology, Karolinska Institute

(Received August 28, 1989)

Murine model of autoimmune diseases

Autoimmune disease is characterized by the appearance of antibodies or cytotoxic lymphocytes directed against self components. The pathogenesis is still unclear but several inbred strains of mice which spontaneously develop autoimmune diseases have recently been developed. These murine models have contributed to our understanding of autoimmune disease from various aspects.[4]

Murphy[5] at the Jackson Laboratory developed one of these strains, called MRL/Mp-*lpr*/*lpr* (MRL/*l*) mice. From about 4 months they show anti-DNA antibody, rheumatoid factor, and anti-Sm antibody (30%) in the serum. They develop lupus nephritis with wire-loop lesions and vasculitis, then die of renal failure at about 8 months. Like human patients the females show more severe symptoms than the males. Systemic lymphadenopathy also occurs. The MRL/Mp−+/+ (MRL/*n*) mouse which lacks the *lpr* (lymphoproliferation) gene has no such systemic lymphadenopathy and develops less severe autoimmune disease, so it is often used as a control.

Serum of the MRL/*l* mouse can induce proliferation of FDC-P2 cells

Recently, we[1] found that the serum of the MRL/*l* mouse could induce the proliferation of the interleukin 3 (IL-3) dependent cell line FDC-P2/185-4, which was originally established by Dexter et al. [6] from the bone marrow cells of the DBA/2 mouse. This cell line was kindly provided by Prof. Kumagai (Tohoku University) and was subcloned at our laboratory.

This activity appeared as early as 1 month, gradually increased with age, and reached a plateau at 4 months in the female. In the male, this proliferative activity appeared somewhat later than 4 months of age.

In the MRL/*n* mouse, this activity appeared after 4 months in the female and did not appear in the male at any time. Thus, this characteristic was considered to be linked with the background genes, of the MRL mouse rather than the *lpr* gene.

To date, no similar effect has been found using serum from normal mice nor from other autoimmune mice, such as the NZB, NZB/NZW F1, and BXSB mice (Fig. 2).

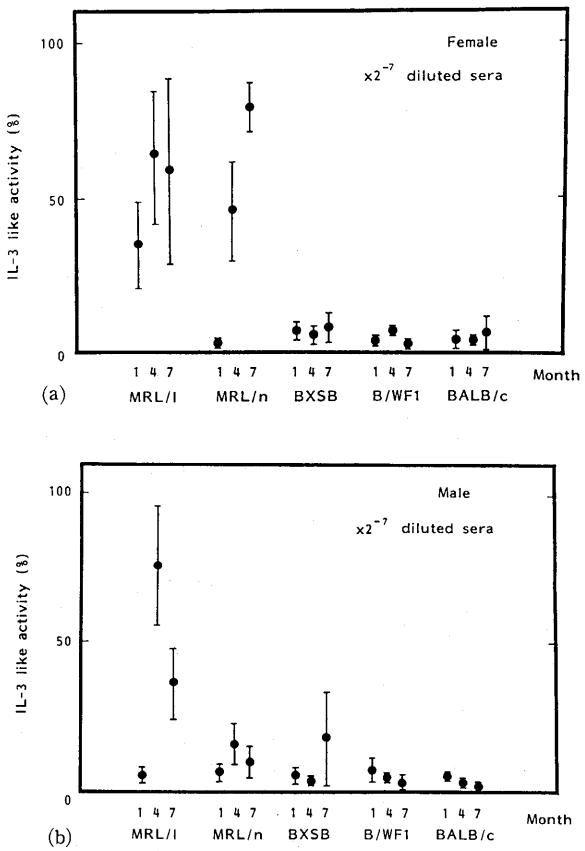

Fig. 2 Murine sera from autoimmune mice at 1, 4 and 7 months of age were diluted 2^7times with RPMI medium containing 5% FCS. The proliferative activity of IL-3-dependent FDC-P2/185-4 cells was measured and was expressed as a percentage of that produced by A4A cell culture supernatant as standard murine IL-3. Each group consisted of 3 mice and each value shown is the mean±SD.[1]
(a) Female
(b) Male

Active component within MRL/*l* serum

At first, we thought simply that the IL-3 level was elevated in the serum of the MRL/*l* mouse. However, Superose gel filtration showed that the activity was not from 28 KD fraction which corresponds to IL-3 but from the 110-150 KD fraction, as shown in Fig. 3.

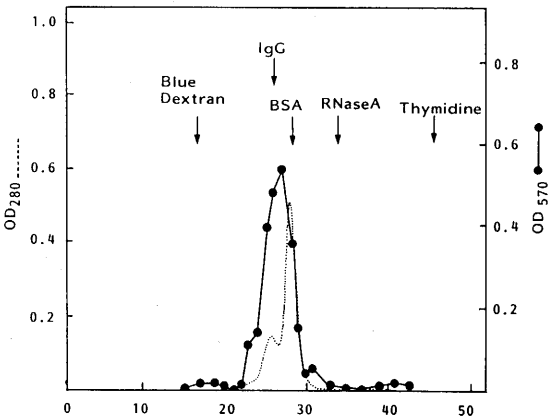

Fig. 3 Elution profile of active fraction from pooled MRL/*l* sera obtained using a Superose 12 gel filtration column.[1)]
●——● ; OD_{570} of MTT assay
---------- ; OD_{280}

We next applied MRL/*l* mouse serum to a Mono Q ion exchange column with a linear gradient of NaCl, and found that the activity was recovered from the fraction eluted with 0.05-0.15 M NaCl and not from the run-through fraction where IL-3 was expected to be (Fig. 4).

From these data we suspected that IgG might be involved.

Therefore, we applied mouse serum to a Protein A Sepharose affinity column and found that the activity was adsorbed to the column and eluted by acid buffer (data not shown). Using an anti-mouse Ig affinity column, we also obtained the same result (data not shown). Thus, we concluded that the activity in the serum was derived from IgG.

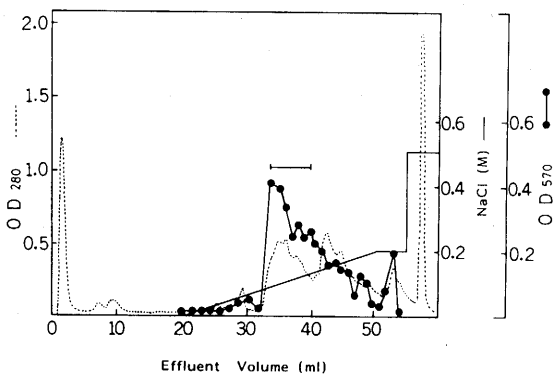

Fig. 4 Elution profile of pooled MRL/*l* mouse sera using an anion exchange Mono Q column with a linear gradient of NaCl.[1)]
●——● ; Proliferative activity expressed by OD_{570} after reduction of MTT
——— ; concentration of NaCl
---------- ; OD_{280}

Not all the IL-3-dependent cell lines respond to MRL/*l* mouse IgG

We next examined the responsiveness of other IL-3-dependent cell lines, such as FDC-P2/K or IC-2, to MRL/*l* mouse IgG. The FDC-P2/K cell is another subclone of FDC-P2 and was kindly provided by Dr. Inaba of the Kansai Medical University. The IC-2 cell [7)] was established by Dr. Koyasu (Tokyo Metropolitan Research Institute) from spleen cells of the DBA/2 mouse. Both are dependent on IL-3 and have been reported to also respond to IL-4 [8)].

When we added serum IgG of the MRL/*l* mouse in various concentration to those cell lines, only the FDC-P2/185-4 cell line proliferated (Fig. 5). It would be of interest to further investigate this difference between the FDC-P2/185-4 and FDC-P2/K cell lines, as both were derived from the same clone originally.

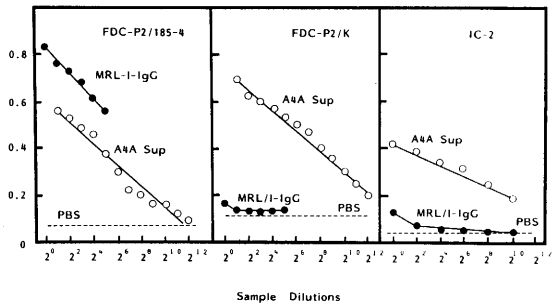

Fig. 5 Responsiveness of three IL-3-dependent cell lines, FD-CP2/185-4, FDC-P2/K, and IC-2 to MRL/*l*-IgG.
Proliferative activity is expressed as the OD_{570} of an MTT assay.[1)]
○ ; A4A culture supernatant as standard IL-3
● ; MRL/*l*-IgG

Mediators of proliferation

We observed that only FDC-P2/185-4 cell line responded to MRL/*l*-IgG among several IL-3-dependent cell lines. However, we next found that even the non-responding FDC-P2/K started to proliferate when grown with culture supernatant from FDC-P2/185-4 cells, stimulated with MRL/*l*-IgG. It appeared that some growth factor might have been secreted by the FDC-P2/185-4 cells after their stimulation with MRL/*l*-IgG. In order to characterize the mediator, we pretreated the supernatant with anti-IL-3 antibody or anti-IL-4 antibody. As demonstrated in Figure 6, the stimulation of FDC-P2/K cells was suppressed by anti-IL-3 antibody.

Thus, it was suggested that IL-3 was secreted by FDC-P2/185-4 cells upon stimulaton with MRL/*l*-IgG. We next added anti-IL-3 antibody to FDC-P2/185-4 cells stimulated with MRL/*l*-IgG to verify the hypothesis that their proliferation was also mediated by IL-3. As we expected, cell proliferation was suppressed by the anti-IL-3 antibody [2)] (data not shown). Thus, it was concluded that the proliferation of FDC-P2/185-4 cells stimulated with MRL/*l*-IgG was mediated by IL-3 secreted by the

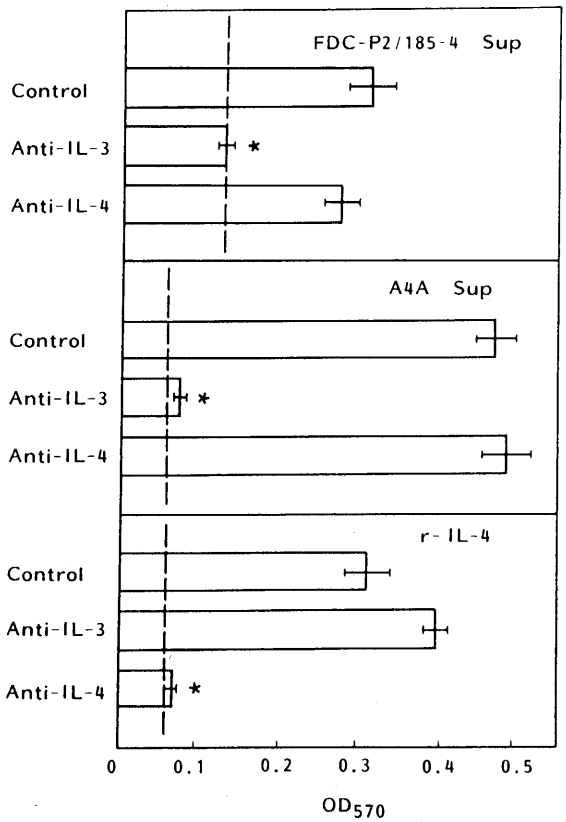

Fig. 6 Effect of anti IL-3 or anti IL-4 antibodies on proliferation of FDC-P2/K cells. Culture supernatant of FDC-P2/185-4 cells (top panel), culture supernatant of A4A cells (middle panel), and recombinant murine IL-4 (bottom panel) were incubated with PBS(-) (control), anti IL-3 antibody (1 : 80 dilution), or anti IL-4 antibody (1:320 dilution) at 37°C for 2 hours. Then, FDC-P2/K cells were added and their proliferation was assayed.[1)]
(---) indicates the degree of reduction of the MTT value in cells cultured only with medium (top panel) or PBS (-) (middle and bottom panel).
*indicates a significant difference ($p<0.001$) compared with each control.

cells themselves, an "autocrine mechanism".

IL-3 was produced by the IL-3 dependent FDC-P2/185-4 cell line upon stimulation with MRL/*l*-IgG at the mRNA level

The m-RNA of murine IL-3 was detected in

FDC-P2/185-4 cells 30 minutes after stimulation with MRL/*l*-IgG by Northern blotting analysis [2] (data not shown).

Signal of MRL/*l*-IgG

We next investigated which part of the MRL/*l*-IgG gave the signal for proliferation. When we added anti-Fc antibody, proliferation was suppressed but when anti-Fab antibody was added no suppression was observed. Thus, it was suggested that the Fc portion of MRL/*l*-IgG played an important role. This concept was further supported by evidence that anti-FcR antibody (2.4G2)[9] could also suppress proliferation[2]. It was concluded that binding between the Fc portion of MRL/*l*-IgG and the Fc receptors of FDC-P2/185-4 cells was required for proliferation. We then asked the question as to whether the Fc portion alone was sufficient for signaling. We produced Fc fragments by digestion of MRL/*l*-IgG with papain and added the fragments to a cell culture. No proliferation was observed (Fig. 7[1]). which suggested that though the Fc portion was necessary, it alone was not enough. Perhaps the Fab portion also plays some role, or else digestion may destroy some necessary three dimensional structure of the Fc portion.

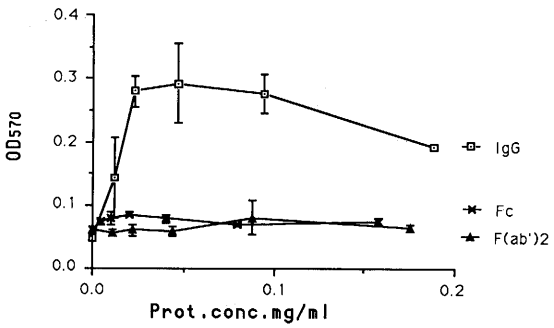

Fig. 7 Proliferation of FDC-P2/185-4 cells induced by whole MRL/*l*-IgG as well as by the $F(ab')_2$ or Fc fragments.[1]

Monoclonal IgG from MRL/*l* mouse

Then we prepared monoclonal IgG with proliferative activity from the MRL/*l* mouse by the cell fusion technique to investigate this antibody more precisely. Two clones were obtained, 1G10 (IgG1, κ) and 1D11 (IgG2b, κ)[2].

There are two possibilities as to why MRL/*l*-IgG can give a signal which normal murine IgG cannot. One is a difference of structure and the other is a special antigen binding capacity. We have purified one of these monoclonal IgGs to homogeneity and investigation of its structure is now in progress. Concerning antigen specificity, we found that 1G10 monoclonal IgG had anti-DNA activity, which is one of the characteristics seen in autoimmune diseases. Recently it has been reported that some anti-DNA antibodies bound to cell surface structures[10]. If this is the case, the 1G10 mAb could bind at two points, the DNA-like structure (s) and the Fc receptor (s) on the cell surface. We are in the process of investigating the recognition molecule (s) on the surface of the FDC-P2/185-4 cells by using Western blotting analysis.

Discussion

Quite recently, we found similar properties in the serum of another autoimmune mouse[3]. Like the MRL/*l* mouse, the IgG of that mouse serum could induce IL-3 from FDC-P2/185-4 cells and cell proliferation also occurred by an autocrine mechanism. Thus, this unique function of IgG is not limited to the MRL mouse. The genetic background of these strains is now under study.

Concerning the proliferation of the FDC-P2 cell line induced by the IgG of the MRL/*l* mouse, Ohta et al.[11]reported that it was due to natural antibody toward the IL-3 receptors of FDC-P2 cells. However, in the cells we are studying this possibility is ruled out, since not all the IL-3 dependent cells can

respond to the MRL/*l*-derived IgG. The mechanisms of the effects reported by Ohta et al, and us must be quite different but probably not mutually exclusive.

As for cytokine production induced by Ig or fragments of Ig or immune complexes, there are several reports. Le Gros et al.[12]reported that immune complexes produced *in vitro* could induce IL-3. However, in our case the active component in the serum was a 110-150 KD protein which corresponds to monomeric IgG. Gel filtration in the presence of 6 M urea also revealed the active fraction at the same region[2], confirming that the active agent is monomeric IgG and not immune complexes.

Thus, the recognition molecule on the cell surface and signal transduction inside the cell are the next subjects to be clarified.

The ability of IgG to give signals towards various cells and to induce various cytokines opens new fields of study both in health and disease.

Acknowledgements

The present work is supported in part by a Research Grant of the Japanese Ministry of Health and Welfare and the Research Project of Yamanashi Medical College. We thank Drs. Kumagai, Ikehara, Abe, Koyasu, Honjo, Inaba and Sudo for providing mice, cells, antibodies, and cDNA probes.

Miss Yuko Nakamura is acknowledged for her excellent secretarial work.

References

1) Tasaka K, Sakihama T, Shirakura-Shibata Y, Matsuda S and Nakajima Y : Proliferative activity of autoimmune MRL mouse serum IgG to interleukin-3-dependent cell through autocrine mechanism. Cellular Immunol. 121 : 317-327, 1989

2) Sakihama T, Akasu F, Iwamoto M, Shirakura-Shibata Y, Nakajima Y and Tasaka K : Polyclonal and monoclonal IgGs from MRL/Mp-*lpr*/*lpr* mice induce an interleukin-3-dependent cell line to produce interleukin-3 through Fcγ-receptor mediated mechanism. Cellular Immunol. in press, 1989

3) Iwamoto M, Akasu F, Sakihama T, Onaya T, Nakajima Y and Tasaka K : Serum IgG from an autoimmune prone mouse C3H/HeJ-*gld*/*gld* supports the interleukin-3-dependent cell line through an autocrine mechanism. Cellular Immunol. in press, 1989

4) Theofilopoulos A N and Dixon F J : Murine models of systemic lupus erythematosus. Adv. Immunol. 37, 269-390, 1985

5) Murphy E D : Lymphoproliferation *(lpr)* and other single-locus models for murine lupus. ed. by Gershwin M E & Merchant B, p. 143-173, Plenum Press, New York 1981

6) Dexter T M, Garland J, Scott D, Scolnick E and Metcalf D : Growth of factor-dependent hemopoietic precursor cell lines. J. Exp. Med. 152 : 1037-1047, 1980

7) Koyasu S, Nakauchi H, Kitamura K, Yonehara S, Okumura K, Tada T and Yahara I : Production of interleukin 3 and γ-interferon by an antigen specific mouse suppressor T cell clone. J. Immunol. 134 : 3130-3136, 1985

8) Grabstein K, Eisenman J, Mochizuki D, Shanbeck K, Colon P, Hopp T, March C and Gillis S : Purification to homogeneity of B cell stimulating factor. J. Exp. Med. 163 : 1405-1414, 1986

9) Unkeless J C : Characterization of a monoclonal antibody directed against mouse macrophage and lymphocyte Fc receptors. J. Exp. Med. 150 : 580-596, 1979

10) Jacob L, Lety M A, Louvard D, Bach J F : Binding of a monoclonal anti-DNA autoantibody to identical protein (s) present at the surface of several human cell types involved in lupus pathogenesis. J. Clin. Invest. 75 : 315-317, 1985

11) Ohta Y, Tamura S, Tezuka E, Sugawara M,

Imai S and Tanaka H : Autoimmune MRL/*lpr* mice sera contain IgG with interleukin 3 like activity. J. Immunol. 140 : 520-525, 1988

12) Le Gros G S, Le Gros J E, Watson J D : The induction of lymphokine synthesis and cell growth in IL-3 dependent cell lines using antigen-antibody complexes. J. Immunol. 139 : 422-428, 1987