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An immunoglobulin-specific autoantibody occurring during alloimmunization suppresses the antibody response

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Abstract. Our previous studies showed that a broadly reactive immunoglobulin G (IgG) anti-immunoglobulin (IgG-anti-Ig) autoantibody is induced during the immune response of LEW rats to BN blood cells. The present experiments analyze the immunoregulatory effect of this physiological autoantibody on antigen receptor-activated B cells in cell cultures. The results show that: (a) At 0.9 pg IgG-anti-Ig/10^6 B cells, an almost complete suppression of the antibody response is induced; we calculated that a few IgG-anti-Ig molecules are sufficient to suppress the antibody response of one B cell; (b) IgG-anti-Ig-induced B-cell suppression is dose-dependent; (c) IgG-anti-Ig suppresses B cells contained in their natural environment (mixed spleen cell population). These data demonstrate that the IgG-anti-Ig autoantibody is an extremely efficient regulatory molecule of the alloimmune response.

Key words: Immunoregulation – Antibody response – Alloimmunization

We have previously shown that an immunoglobulin G (IgG)-anti-immunoglobulin autoantibody (IgG-anti-Ig) appears in the serum of alloimmunized rats in addition to the donor-specific antibody [4]. Unlike antiidiotypes, this antibody recognizes a conserved domain of the IgG molecule. The current series of experiments addressed the question of whether this antibody has an immunoregulatory function.

Materials and methods

IgG-anti-Ig antibody. LEW rats were repeatedly transfused with 1 ml BN blood cells [5], and the sera of immunized animals with a high IgG-anti-Ig antibody titer were collected. The IgG fraction was separated by protein G chromatography and gel filtration, and anti-Ig was extracted by affinity chromatography on immunoglobulin-coupled sepharose [6].

B-cell separation. Mononuclear spleen cells (5×10^6) of LEW rats separated by density gradient centrifugation were incubated sequentially with 5 µl of mouse anti-rat lymphocyte monoclonal antibodies (Serotec, Oxford, UK) [against T cells, stem cells, plasma cells, Th cells, macrophages, Ts cells, cytotoxic T cells, natural killer (NK) cells], 6 µl goat F(ab')2-anti-mouse IgG(Fc)-biotin, 3.7 µg avidin, 0.25 µl biotin-labelled magnetic ferrit-polyglucose particles and passed twice through a ferromagnetic separation column (Miltenyi Biotec, Bergisch-Gladbach, FRG).

Cell cultures. B cells (10^6 or mononuclear LEW spleen cells 2×10^6) were stimulated with 23 µg of goat F(ab')2-anti-rat IgM and supernatant of concanavalin A (ConA)-activated LEW lymphocytes in serum-free medium as previously described [7]. Increasing amounts of affinity purified IgG-anti-Ig were added to the culture. Three days later, the antibody production of B cells was measured in a reverse plaque-forming cell assay (PFC/10^6 cells; $\bar{x} \pm$ SEM) [6]. Stimulated cells served as the positive control (100% response) and unstimulated cells, as the negative control (0%).

Results

As increasing amounts of IgG-anti-Ig antibody were added to the culture, the B-cell response gradually decreased (Table 1). At 0.9 pg IgG/10^6 cells, an almost complete suppression was obtained. By increasing the antibody concentration further, the suppressive effect disappeared. IgG obtained from nonimmunized LEW rats ("irrelevant" control IgG) had no effect.

In the absence of T cells, B cells are more sensitive to IgG-induced suppression [3]. In vivo the B cells are part of a mixed cell population including T cells. To establish whether the regulatory antibody also suppresses B cells in their natural environment, spleen lymphocytes were cultured in the presence of suppressive antibody. As shown in Table 2, a similar dose-response curve as that obtained with purified B cells was obtained. The "irrelevant" control IgG had no effect.

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Table 1. Suppression of B cells by an affinity-purified IgG-anti-Ig autoantibody

Amount (pg IgG/10^6 cells)	B-cell response (%)	
	Irrelevant IgG (control)	IgG-anti-Ig
0.03	106	120
0.06	101	73
0.1	94	54
0.2	114	57 ± 17
0.5	102	48 ± 13
0.9	95	15 ± 15
1.9	107	39 ± 2
3.7	112	39 ± 0.7
7.5	114	40 ± 4
15	115	78 ± 17

Ig, immunoglobulin
Increasing amounts of "irrelevant" control LEW IgG or LEW IgG-anti-Ig were added to antigen receptor-activated purified B cells derived from LEW rats. The B cells' antibody production was determined in a reverse plaque-forming cell assay ($\bar{x} \pm$ SEM of PFC/10^6 cells) after 3 days of culture. The positive control consisted of stimulated B cells (6962 PFC/10^6 cells = 100%) and the negative control of unstimulated B cells (1088 PFC 10^6 cells = 0%). Maximum suppression was obtained at 0.9 pg IgG/10^6 cells

Table 2. Suppression of spleen lymphocytes by an affinity-purified IgG-anti-Ig autoantibody

Amount (pg IgG/10^6 cells)	B-cell response (%)	
	Irrelevant IgG (control)	IgG-anti-Ig
0.01	89	81 ± 7
0.02	94 ± 11	55 ± 6
0.05	98	26 ± 13
0.1	93 ± 5	10 ± 4
0.2	105 ± 9	7 ± 7
0.4	102 ± 2	25 ± 15
0.8	104 ± 11	25 ± 12
1.7	93 ± 7	54 ± 21
3.4	103 ± 5	94 ± 12

Increasing amounts of "irrelevant" control LEW IgG or LEW IgG-anti-Ig were added to antigen receptor-activated LEW spleen lymphocytes. After 3 days the antibody production was determined in a reverse plaque-forming cell assay ($\bar{x} \pm$ SEM of PFC/10^6 cells). The positive control consisted of stimulated cells (8189 PFC/10^6 cells = 100%) and the negative control of unstimulated cells (712 PFC/10^6 cells = 0%). Maximum suppression was obtained at 0.2 pg IgG/10^6 cells

Discussion

The IgG-anti-Ig produced during alloimmunization suppresses the B-cell response. The antibody is effective at extremely small concentrations. Based on the molecular weight of IgG and Avogadro's number, our results indicate that a few antibody molecules are sufficient to suppress the activity of one B cell.

We have reported previously that the mechanism of B-cell suppression by IgG-anti-Ig is Fc receptor-dependent [7]. Others have shown that heterologous Ig-specific antibodies crosslink the B cells' antigen receptor with its Fc receptor and that this crosslinking leads to an inactivating signal [2, 1]. In our test system, it is likely that the IgG-anti-Ig autoantibody induces suppression by the antigen receptor/Fc receptor crosslinking. It is known that anti-immunoglobulins have different affinities for the antigen receptor and the Fc receptor [9]. This provides an explanation for our finding that suppression is obtained only at a certain antibody concentration. Whereas an optimum concentration leads to co-crosslinking of the two receptors, higher concentrations may affect only one of the two receptors. An alternative explanation for the suppressive mechanism is the independent occupation of the Fc receptor (by IgG + anti-IgG immune complexes) and the antigen receptor (by its ligand) followed by their cocapping. The resulting sterical adherence of the two receptors leads to an inactivating signal [8].

Unlike our previous experiments [6] in which mitogen-stimulated B cells were studied, the current study analyzes the effect of IgG-anti-Ig on antigen receptor-activated B cells. In addition to this "physiological" B-cell activation, the relevance of our test system for the situation "in vivo" was increased by using antibody and lymphocytes derived from the same rat strain.

Our findings show that the IgG-anti-Ig antibody induced during the alloimmune response is a highly active, self-regulatory molecule of the immune system.

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