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Immune Phagocytosis Inhibition by Commercial Immunoglobulins

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Summary. Sixteen commercially available immunoglobulins (Ig) and 5 anti-Rho (D) hyperimmune globulins were investigated for immune phagocytosis inhibition (IPI) factors as well as for T, B lymphocytotoxic and monocytotoxic antibodies. All Ig contained IPI factors with lowest inhibitory IgG concentrations ranging from 0.08 to 50 mg/ml. Pepsin-digested Ig was noninhibitory. IPI factors in anti-D preparations were uniformly high (inhibitory IgG concentrations 0.6–2.5 mg/ml). Cytotoxic antibodies against T, B lymphocytes and monocytes were found in 2,2 and 7 products, respectively. Since we have recently shown that IPI is caused by antibodies against major histocompatibility complex antigens, most likely HLA, the hypothesis is put forward that IPI factors in Ig are HLA-related, cytotoxic as well as noncytotoxic antibodies which act via Fc receptor blockade of human monocytes.

Key words: Immunoglobulin – ITP – Immunphagocytosis

High dose intravenous immunoglobulin G (IgG) has been shown to be an effective treatment of autoimmune thrombocytopenia [10], posttransfusion purpura [1a], autoimmune neutropenia [16, 6] and a number of other conditions (for a review: McClelland and Yap, 1984 [12]. A similar effect can be achieved in Rhesus-positive patients with autoimmune thrombocytopenia by the administration of small doses of anti-D IgG [1, 17, 17a]. There is experimental evidence that the effect of IgG is mediated by a transitory blockade of the reticuloendothelial system (RES) [9]. Its mechanism, however, has remained unknown. We have recently reported that immune phagocytosis of anti-D sensitized human red blood cells (anti-D-RBC) by human peripheral blood monocytes can specifically be inhibited in vitro by monoclonal as well as polyclonal antibodies directed against certain MHC products [13]. This immune phagocytosis inhibition (IPI) was not due to cell injury or paralysis since particle phagocytosis was unaffected. As IgG is prepared from large plasma pools it is conceivable that it contains antibodies (e.g. from HLA sensitized individuals) capable of inducing the IPI

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phenomenon. We here will show that all unmodified commercial IgG products as well as anti-D hyperimmunoglobulins tested do indeed contain IPI-positive antibodies. We hypothesize that this phenomenon might be related to the in vivo effect of IgG or anti-D-IgG and may explain, at least in part, their effect on RES function.

Materials and Methods

Cells: Human mononuclear blood cells (MNC) were isolated on a discontinuous gradient [3] and separated into T and B lymphocytes by nylon wool adherence [11]. Monocytes were isolated by adherence in the wells of Terasaki microtiter plates for the monocyte cytotoxicity test [14], or on microscopic glass slides for the IPI-Test [13].

IgG: Commercially available products of IgG as well as anti-D-IgG were purchased from or were kindly provided by the manufacturers. Pertinent data are given in Table 1. All specimens were adjusted to an IgG concentration of 50,000 μg/ml and were then serially diluted for testing.

Procedures: The T lymphocyte cytotoxicity test (TCT) was performed by the standard NIH microlymphocytotoxicity test [20]. The B lymphocyte cytotoxicity test (BCT) was done accordingly with prolonged incubation times (1 and 2 h). The monocyte cytotoxicity test (MCT) on adherent cells using carboxyfluorescein for live and ethidiumbromide for dead cells has been described previously [14] and proved to be more sensitive and less unspecific than tests on monocytes in suspension. The reactions of the TCT, BCT and MCT were scored in accordance with the 8th International Histocompatibility Workshop 1980, Los Angeles [21]. The IPI test has already been described in detail [13]. Briefly, about 2,000 monocytes adherent to glass slides were preincubated with 30 μl IgG solution at 37° for 60 min, then thoroughly washed, overlaid with 30 μl anti-D-RBC (8×10^9/ml) and incubated at 37° for 60 min. After another wash the specimens were dried, stained (Wright-Giemsa) and evaluated microscopically. For each preparation, 100 monocytes were counted and the percentage of monocytes having ingested one or more RBC was determined. Normal values ($\bar{x} \pm 1$ SD) were 59% ± 6.6% (N = 15). From these figures "relative inhibition" was calculated as reported [13] and used for the definition of IPI in that ≥ 35% of phagocytosing cells were considered as absence, < 35% as presence of inhibition. In most assays which revealed inhibition less than 1% of phagocytosing cells were observed.

Results

IPI inducing factors showed remarkable differences of titer among commercially available IgG products with lowest inhibitory IgG concentrations ranging from 50 to 0.08 mg/ml (Fig. 1, No. 2–16). Pepsin-treated IgG (No. 1) was not inhibitory at all. IPI titers in anti-D-IgG products (No. 17–21) were uniformly high, their inhibitory IgG concentrations varied between 2.5 and 0.6 mg/ml. The highest content of IPI factors was detected in an IgG preparation derived from human placentae by acidic elution (No. 16). This product had also high cytotoxicity titers to T, B lymphocytes and monocytes. Cytotoxicity to all mononuclear cells was detected in product No. 15 at low concentrations (Fig. 1). Exclusive cytotoxicity to monocytes was found in four IgG and in one anti-D product. In controls with inactivated sera substituting for complement no cytotoxicity was detected (data not shown).

Table 1. Immunoglobulins used in the study

No.	designation	batch analyzed	product of	characteristics of preparation/treatment	mode of administration
1	Gamma-Venin	420521	Behringwerke, Marburg, FRG	pepsin	i.v.
2	Intraglobulin	401113	Biotest, Frankfurt, FRG	β-propiolactone	i.v.
3	Venimmun	421036	Behringwerke, Marburg, FRG	sulfitolysis	i.v.
4	Rhodiglobin	2 VN 1120 X 0080	Institut Mérieux/Rhone-Poulenc, Norderstedt, FRG	plasmin	i.v.
5	Intraglobin F	414054	Biotest, Frankfurt, FRG	β-propiolactone	i.v.
6	Sandoglobulin	101, 30.06.85	Sandoz, Nürnberg, FRG	pH 4	i.v.
7	Polyglobin	01404847	Cutter/Troponwerke, Köln, FRG	reduction, alkylation	i.v.
8	Gammagard	830118 AG 11 A	Travenol, München, FRG	DEAE-sephadex	i.v.
9	Endobulin	2410483	Immuno, Heidelberg, FRG	PEG-HES	i.v.
10	Gammonativ	80794	KabiVitrum, München, FRG	DEAE-sephadex	i.v.
11	Beriglobin	401268	Behringwerke, Marburg, FRG		i.m.
12	IVIG HORM	407244	Hormon-Chemie, München, FRG		i.v.
13	Pentaglobin	461104	Biotest, Frankfurt, FRG		i.v.
14	Immunglobulin 7S human i.v.	2162/1	Armour Pharma, Eschwege, FRG	PEG-HES	i.v.
15	Cutterglobin	0070284	Cutter/Troponwerke, Köln, FRG		i.m.
16	Arthroglobine[a]	S 1381	Institut Mérieux, Lyon, France	IgG from acidic eluate of placentae	i.v.
17	Partobulin	18118408	Immuno, Heidelberg, FRG		i.m.
18	HypRho-D	C 51793	Cutter/Troponwerke, Köln, FRG		i.m.
19	Rhesonativ 300	81270	KabiVitrum, München, FRG		i.m.
20	Rhesogam	406562	Behringwerke, Marburg, FRG		i.m.
21	Immunglobulin G Anti-Rh	195	Central Institute of Blood Transfusion, Hamburg, FRG	DEAE-sephadex	i.v.

[a] not commercially available

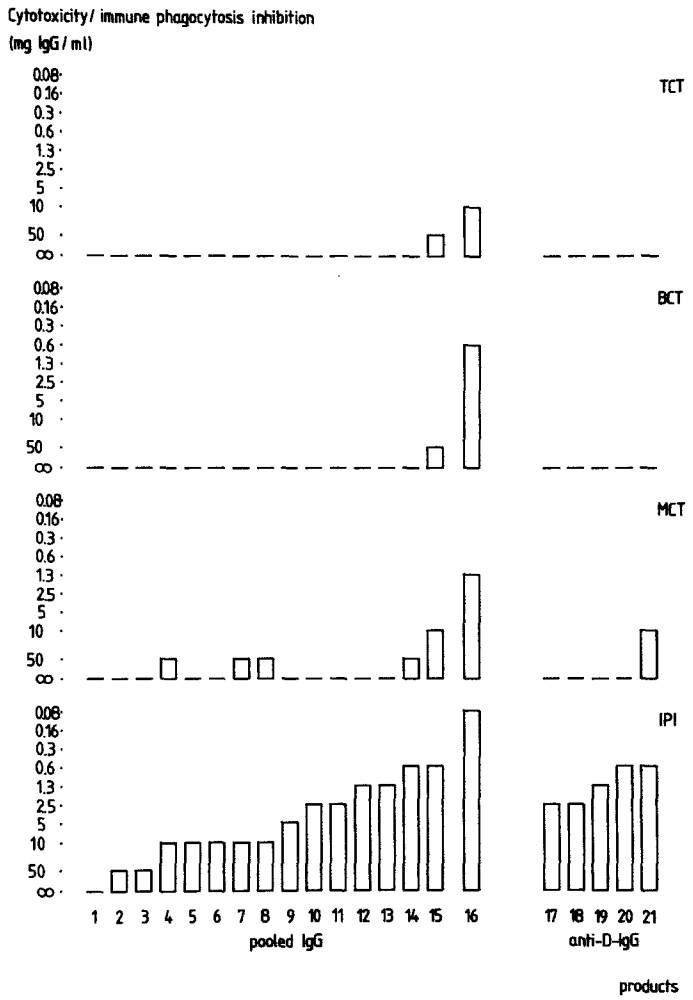

Fig. 1. Comparison of commercial IgG and anti-D products: T lymphocyte cytotoxicity test (*TCT*), B lymphocyte cytotoxicity test (*BCT*), monocyte cytotoxicity test (*MCT*) and immune phagocytosis inhibition (*IPI*), expressed as lowest IgG concentration which still excerts cytotoxicity or inhibition. For explanation of the products see Table 1. If the results at a concentration of 50 mg/ml were negative, the column was omitted (∞)

Discussion

As we have recently reported, murine monoclonal antibodies specific for HLA determinants as well as human alloreactive antisera are capable of inducing IPI [13]. This effect depends on the integrity of antibody molecules in that only complete immunoglobulin molecules which have bound specifically to monocytes by their F(ab)2 portion and unspecifically by their Fc portion are active in IPI [13]. Our results indicate that similar IPI factors are present to a varying extent in all commercial IgG and anti-

D products except one pepsin-digested preparation. Although the nature of these factors is as yet unknown there is strong evidence that they represent noncytotoxic antibodies to HLA or HLA-related monocyte antigens.

Since commercial IgG products are usually manufactured from large plasma pools it is conceivable that the marked differences of IPI factor concentrations are related to the various plasma sources differing in their proportion of presensitized individuals (e.g. by pregnancies). This view is further corroborated by our observation that IPI titers of anti-D hyperimmunoglobulins were generally higher than those of random IgG probably reflecting the exclusive origin of anti-D from individuals immunized with human red blood cells. Further indirect arguments for IPI factors as the result of immunization were that at least four IgG and one anti-D preparation had monocytotoxic, complement-dependent activity and that one IgG preparation (No. 15) exhibited cytotoxicity towards T, B lymphocytes and monocytes. The highest content of IPI factors was detected in IgG No. 16 derived from placentae by acidic elution [4, 5]. This preparation not only showed the highest cytotoxicity titers, but had a marginal IPI factor concentration of 0.08 mg/ml which almost exactly corresponded to that of the B lymphocyte rosetting inhibition test (0.1 mg/ml) as described by Clot et al. [7]. Finally, our finding that the only IPI negative preparation was a pepsin-digested product confirms that the Fc portion of immunoglobulins is a prerequisite for their effect [13]. This is in contrast to recent findings [19]: Using the erythrocyte antibody rosette inhibtion technique with B lymphocytes in this study no difference of inhibitory capacity was detected amoung various commercial IgG products (including Fc deprived F(ab)2-fragments).

The biological significance of IPI antibodies may rest in their capability to inhibit primary or secondary immune responses at the level of antigen ingestion by macrophages. With respect to immunization through pregnancy one could speculate that maternal IgG antibodies after their placental transfer could not only opsonize fetal target cells but similarly inhibit monocyte phagocytosis thus compensating for their harmful effect. Petri et al. [15] have already discussed the possibility that Fc receptor blocking antibodies in anti-D-IgG could ameliorate the course of hemolytic disease of the newborn, an effect also reported with high-dose IgG [2]. We here propose that IPI antibodies, most likely noncytotoxic antibodies specific for HLA or related determinants and present in all unmodified immunoglobulin preparations, might explain, at least in part, the beneficial therapeutic effect of placenta-eluated IgG in rheumatoid arthritis [8, 18,], of high-dose IgG [10] or anti-D-IgG [17] in autoimmune thrombocytopenia, and of anti-D in prevention of Rhesus incompatibility.

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