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The Biological Properties of Immunoglobulin G and its Split Products [$F(ab')_2$ and Fab]

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Summary. Antibodies of the IgG class possess antibacterial, antiviral and toxin neutralizing properties and for this reason are administered prophylactically and therapeutically. In the case of the immunoglobulin preparations commercially available for i.v. application a basic distinction must be made between unsplit immunoglobulins and those antibody preparations obtained by enzymatic digestion, such as $F(ab')_2$ or Fab antibodies. This survey deals with the largely experimental evidence describing the biological properties of these preparations. Administration of antibodies in the presence of the corresponding antigens leads to the formation of immune complexes in the organism. These immune complexes can activate, either directly or indirectly, the cellular and humoral systems which are involved in phagocytosis and the elimination of antigens, in the regulation of the body's own antibody production and in inflammatory reactions. As a result of their inability to interact with Fc receptors, immune complexes with $F(ab')_2$ or F(ab) antibodies appear to be less active in the release of inflammation mediators from leucocytes and thrombocytes than immune complexes with unsplit immunoglobulins. These, on the other hand, can antigen-specifically and non-antigen-specifically suppress the immune system which is not the case for immune complexes with $F(ab')_2$ or Fab antibodies. There are indications that these split products also occur in vivo due to the action of tissue and leucocyte proteases. Unlike Fab preparations, $F(ab')_2$ antibodies have antibacterial and antiviral potencies similar to unsplit immunoglobulins, which is probably due to the ability of $F(ab')_2$ molecules to activate complement, not by the classical but by the alternative pathway. Like Fab preparations, $F(ab')_2$ molecules appear to be superior to unsplit IgG in the elimination of haptens. On account of the relatively long period of time unsplit immunoglobulins remain in the blood, they are well suited for prophylactic treatment and substitution over longer periods. The extent to which indications, obtained predominantly from experimental studies, of a reduced release of inflammation mediators, a lack of immune suppression and a lack of augmentation of IgG catabolism would advocate the use of $F(ab')_2$ split products, especially for therapeutic purposes, can only be ascertained after prospective and comparative studies have been carried out.

Key words: Immune complexes – i.v.-immunoglobulin preparations – 7S-IgG – $F(ab')_2$ – Fab – Inflammation – Side-effects – Therapy – Prophylaxis

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Introduction

Ever since Emil von Behring began his serum therapy (von Behring and Kitasato 1890), the effect of specific antibodies for the prophylaxis and treatment of infectious diseases has been well known. Since this time, however, there has been an awareness of the serious acute and subacute side-effects linked with the administration of heterologous antibody preparations. In order to reduce these side-effects, heterologous "fermosera" were produced by means of an enzymatic removal from the antibody of the Fc part which is not directly involved in antigen binding. These fermosera proved to be as effective as the traditional "intact" immunoglobulin preparations while being better tolerated (Weil et al. 1938; Schmidt 1939; Arlt 1940). In spite of this enzyme treatment the fermosera, as a foreign protein in the recipient, induced the for-

mation of specific antibodies and thus caused the so-called serum diseases; this was, however, to a lesser degree than that caused by unsplit immunoglobulines (Schmidt 1939; Arlt 1940). This risk factor could not be eliminated until homologous antibody preparations were used (Stokes 1951). Nowadays a great many homologous antibody preparations are available; here one must distinguish between the so-called polyvalent antibody preparations, containing the antibody spectrum of a donor population without specific enrichment, and the specifically enriched antibody preparations. Moreover, a distinction must be made between preparations intended for intramuscular and i.v. injection (Barandun 1964; Schneider and Weitzel 1974; Schultze and Schwick 1962). Since intramuscular injection is only possible in limited amounts and at limited intervals, large amounts must be administered prophylactically and/or therapeutically by means of i.v. infusion.

Two groups of commercially available antibody preparations for i.v. administration should be distinguished from one another. Unsplit immunoglobulins of the IgG class, the tolerability of which for i.v. administration has been improved by means of a careful fractionating process or some sort of chemical treatment, and antibody preparations produced by means of enzymatic splitting of IgG. The latter fall into two groups which contain largely $F(ab')_2$ molecules [IgG-$F(ab')_2$] or Fab antibodies [IgG-Fab], respectively.

It is the aim of this survey to summarize the most important studies on the biological effects of unsplit IgG antibodies, $F(ab')_2$ and Fab molecules and, on the basis of this information, to discuss their possible significance for clinical purposes. It is not the aim of this paper to deal with the various processes and problems in the production and testing of tolerable unsplit immunoglobulin preparations. This question has already been discussed in detail elsewhere (Seiler et al. 1980; Gronski and Seiler 1983).

Occurrence

The concentration of IgG in the blood and the period of time it remains there have been ascertained by various methods. They constitute physiological parameters with the according fluctuation range. They depend on the amount of antibody formed per time unit in answer to the respective antigen stimulation (with this mechanism being subject to its own feedback control), the serum concentration – which determines the degree and the speed of the antibody catabolism, the distribution into extravasal spaces and the consumption by specific antibody functions. In the case of infections consumption will be increased, in addition to the specific binding of antibodies, by the influence of proteases such as cathepsins, elastases or plasmin on immunoglobulins (Lo Spalluto et al. 1971). These proteases can be released from various tissues (connective tissue, muscle, spleen or liver tissue) through cell destruction, or they can be released, in the course of an inflammation, from lysosomes of granulocytes or macrophages. They may also arise during the activation of the blood clotting system, as is known in the case of plasmin.

Some of the cathepsins, especially cathepsin D, have a similar effect to that of pepsin (Mycek 1970; Ghetie and Mota 1971); that is to say that during proteolysis, primarily $F(ab')_2$ molecules arise locally from IgG (Menninger et al. 1976; Fehr et al. 1974; Lo Spalluto et al. 1971). It is known that plasmin and elastase can split a large part of IgG into Fab and Fc fragments (Prince et al. 1979a; Ghetie and Sulica 1970). IgM can be split into $F(ab')_2$ molecules by lysosomal elastase of human granulocytes (Prince et al. 1979b). Both $F(ab')_2$ and Fab molecules may thus be regarded as immunoglobulin split products which appear naturally in the body. A further indication of this is the fact that in sera from patients with rheumatoid arthritis or from patients suffering from a chronic infection, and also occasionally in the sera of healthy persons, another type of IgG antibody is sometimes found – in addition to or instead of rheumatoid factors (antibodies directed against the Fc part of IgG) – which reacts only with isolated $F(ab')_2$ and not with intact IgG. These so-called pepsin agglutinators seem to be involved in the enhancement of the elimination of antigen-bound $F(ab')_2$ antibodies (Osterland et al. 1963; Natvig 1970; Johnson and Page-Faulk 1976) and thus to complement the role of the rheumatoid factors in the "physiological" elimination reaction (Johnson and Page-Faulk 1976; Masson 1976).

Half-Life Time and Catabolism

As mentioned above, the concentration of IgG and the duration of its intravasal presence are a result of the rate of antibody synthesis on the one hand, and the degradation rate or the distribution speed on the other (Waldmann and Strober 1969). As the body tries to maintain a steady state of IgG, the administration of immunoglobulin will influence this balance in varying degrees, either through an inhibition of the specific antibody formation or through an increase in the catabolic rate. This will depend on the type of preparation administered and on the clinical situation. It is known

that the infusion of unsplit IgG preparations leads to an increase in the catabolic rate for IgG (Schnoz 1979; Ryser 1979). This effect is dependent on the Fc part of immunoglobulin; it can even be achieved under experimental conditions by the Fc fragment alone (Waldmann and Strober 1969; Fahey and Robinson 1963). By contrast, in the studies cited no influence on the catabolism of IgG could be proven when Fab or $F(ab')_2$ molecules were injected.

The increased catabolic rate contributes to the half-life of an immunoglobulin preparation. Moreover, the degree of denaturation during the course of its isolation or subsequent chemical modifications are of decisive importance (Bocci 1976). However, it is clear that the length of time immunoglobulin remains in the blood depends predominantly on the Fc part. Fab or $F(ab')_2$ molecules have a relatively short half-life (IgG-Fab, 3–5 h – Waldmann and Strober 1969; IgG-$F(ab')_2$, about 48 h – Morell and Skvaril 1980) while the half-life of unsplit immunoglobulins is about 20 days (9–28 days – Waldmann and Strober 1969). The half-life of chemically modified immunoglobulins is in most cases somewhat lowered (Morell and Skvaril 1980).

Tissue Distribution

Approximately 50% of IgG is extravasal (Taliaferro and Talmage 1956; Waldmann and Strober 1969) and thus predominantly in the interstitium. There are, however, various indications that IgG is also found intracellularly. Thus Mayersbach (1957) and Mitrenga et al. (1975) were able to show the presence of human IgG in the cytoplasma of mouse hepatocytes after i.v. infusion; also, Alarcon-Segovia et al. (1978, 1979a, b and c) demonstrated, in the homologous human system that antibodies penetrate living cells in vivo and can, for instance, be present as auto-immune antibodies of pathophysiological importance. Vollerthun et al. (1977) and Vollerthun and Möller (1980) demonstrated in vivo and in vitro in the heterologous systems of the mouse and chicken that $F(ab')_2$ molecules penetrate quicker and in higher concentrations the interstitium and the cells than unsplit immunoglobulin after i.v. administration. These findings could be confirmed by Kitagawa and Okumura (1980), Enokidani et al. (1981), Llerena et al. (1981) and Kaneko et al. (1981). At present there is thus no doubt about the ability of antibodies to penetrate cells or the quicker tissue distribution of $F(ab')_2$ fragments. Reliable comparative studies on humans are so far not available. Bauer et al. (1979) were not able to demonstrate the presence of IgG or $F(ab')_2$ molecules after administration of an i.v. infusion in the human intestinal mucous membrane. This was possibly due to methodological reasons since, during immunohistological tests on quick-frozen sections, both intercellular and intracellular immunoglobulin is removed if washing is performed before staining. Thus information has only been available from non-comparative studies which maintain that in humans $F(ab')_2$ antibodies are demonstrable in liver cells (Ronneberger et al. 1978) or in the peritoneal exudate (Dethlefsen and Stübner 1980) as functioning, that is to say, antigen binding molecules shortly after i.v. injection. It is, however, unclear whether there are any advantages in an increased cellular penetration of antibody preparations.

Antigen-Specific and Antigen-Unspecific Immune Suppression

For some time it has been known that the administration of immunoglobulins can lead to a suppression of the natural formation of antibodies of the same specificity in the body (Uhr and Baumann 1961; Bystryn et al. 1970). While subsequent tests with $F(ab')_2$ molecules produced contradictory results at first [possibly due to the difficulty of preparing IgG-$F(ab')_2$ of the necessary purity], the tests – both in vitro and in vivo – carried out by various groups during the past few years have demonstrated quite clearly that the antigen-specific suppression is dependent on the Fc part of the IgG antibodies (see Table 1). The binding of immune complexes to the corresponding antigen and Fc receptors of specifically committed lymphocytes causes a blockade of these cells and thus results in an inhibition of antibody synthesis. There are no indications that $F(ab')_2$ or Fab molecules are specifically immune suppressive, if one discounts the exception of the keyhole-limpet haemocyanin antigen (Cerottini et al. 1969; Abbas and Klaus 1978). On the contrary: it appears possible that $F(ab')_2$ antibodies stimulate the immune system antigen specifically (Morgan and Tempelis 1978). There is similar information about IgM (Forni et al. 1980).

Antigen-antibody complexes can have immune suppressive effects due to an interaction with B-lymphocytes (Oberbarnscheidt and Kölsch 1978) or to the influence on T-B cell cooperation (Hoffmann and Kappler 1978). On the other hand, immune complexes can also have immune stimulatory effects. This activity depends on the antigen in question, on the quantity and quality of the

Table 1. Antibody mediated specific suppression of the antibody response

Authors	Antigen	Animal	Inhibition		Stimulation
			+	−	+
Uhr & Baumann 1960	Diphteriatox. BSA OV-Alb Bovine-IgG	Guinea-pig Rabbit Rat (in vivo)	IgG		
Dixon et al. 1967	KLH	Rabbit	IgG		
Cerottini et al. 1969	KLH	Rabbit	IgG $F(ab')_2$ Fab		
Abbas & Klaus 1978	DNP-KLH	Mouse (Plasmazytoma (in vitro)	IgG $F(ab)_2$		
Tada 1971	DNP-Ascaris	Rat	IgG, IgE		
Sinclair et al. 1968	SRBC	Mouse	IgG	$F(ab)_2$	
Sinclair 1969	SRBC	Mouse	IgG	$F(ab)_2$	
Sinclair et al. 1970	SRBC	Mouse	IgG	$F(ab)_2$	
Dennert 1970	SRBC	Mouse (in vitro, in vivo)	IgG		IgM
Stockinger & Lemmel 1978	SRBC	Mouse	IgG $F(ab)_2$ + Anti-$F(ab)_2$-IgG	$F(ab')_2$	
Hoffmann & Kappler 1978	SRBC, TNP	Mouse (in vitro)	IgG $F(ab)_2$ + T- helper factor	$F(ab')_2$	
Oberbarnscheidt & Kölsch 1978	SRBC, HRBC	Mouse (in vitro)	IgG	$F(ab)_2$ $F(ab)_2$ + LPS $F(ab)_2$ + TRF	
Morgan & Tempelis 1978	BSA, SRBC	Chicken	IgG	$F(ab)_2$	F(ab)
Feldmann & Diener 1972	Flagellin	Mouse	IgG	$F(ab)_2$	Fab
Tite & Taylor 1979	NAP + AK	Mouse	IgG	$F(ab)_2$	
Tony & Schimpl 1981	IgG	Mouse	IgG		F(ab)

antibody and on the antigen-antibody ratio in the immune complexes involved. Thus immune stimulatory effects of immune complexes arise mostly in antigen excess, and immune suppressive effects in excess of the antibody (see survey: Sedlacek 1980a and b).

The clinical significance of experimental data on antigen-specific immune suppression is not as yet clear. It is not clear, for example, as to whether the suppression of sensibilisation against rhesus factor positive erythrocytes by the administration of anti-D antibodies is a process which belongs to antigen-specific immune suppression by immune complexes. Since antigen-specific immune suppression is an immunologically active process, one could suppose that, clinically, it could only take effect after the administration of gammaglobulin preparations in patients whose immune systems function more or less normally, but not in patients with a primary or secondary impairment of their immune system (for instance after cytostatic treatment).

Besides an antigen-specific immune modulation by means of immune complexes, there are indications that immunoglobulins can also affect the immune system antigen unspecifically. Thus a stimulation of the antibody response has been observed in vivo for $F(ab')_2$ molecules (Kulberg et al. 1976 and 1978), while unsplit IgG causes more of an immune suppressive effect. Thus a substitution with large amounts of an unsplit immunoglobulin (treated with β-propiolacton) during plasma exchange treatment caused a decrease of the IgM, IgA and IgG immunoglobulin levels in comparison to control patients who were substituted with an albumin solution or "fresh-frozen" plasma (Beyer

et al. 1980). Moreover, Fateh-Mogadham et al. (1982) were able to demonstrate, in the case of myasthenia gravis, that the infusion of greater amounts of unsplit immunoglobulins, but not of $F(ab')_2$ molecules, caused an absolute reduction of the auto-antibody level and an improvement of the clinical symptoms.

Effectiveness

The most striking property of antibodies is their antibacterial, antiviral and toxin neutralizing effectiveness. On account of this they are used prophylactically and therapeutically in the treatment of contagious diseases. Thus methods for the isolation, splitting or chemical modification of immunoglobulins should not impair these basic properties. They should be controlled using suitable pharmacological models and they must also be confirmed in clinical studies. In vitro studies can only produce very limited evidence concerning effectiveness. This is on account of the complex cellular and humoral processes taking place during the resistance to bacteria, viruses and the elimination of toxins in which antibodies do indeed play an important role but they nevertheless represent only one of the many partners. The results of relevant in vivo animal models, i.e. ones which resemble the clinical situation (in vivo), will thus be of decisive importance. These, however, are often extremely difficult to assemble. Moreover a comparison with a standard preparation is necessary to test the effectiveness of a modified or split immunoglobulin preparation. This standard preparation must be produced from the same immune serum or pool as the preparation to be tested since, despite all possibilities of comparison, antibody preparations, especially in the case of small donor populations and, depending on the immunising phase, can be heterogeneous in respect of the proportion of specific and cross-reacting, weakly and strongly binding (affined) antibodies.

Compared with pure $F(ab')_2$ and Fab preparations there have been very few animal experiments in which unsplit immunoglobulin was tested under such stringent conditions. Ax et al. (1981) tested the influence of various antibody split products, produced from rabbit hyperimmune serum, on the elimination of bacteria and the survival rate subsequent to E. coli infection in mice. Trautmann et al. (1982) carried out experiments under similar conditions in the case of experimental pseudomonas sepsis. Stephan (1980) established the survival rate of mice which were infected through the nose with a mixture of influenza A_2 Taiwan viruses and various antibody preparations. In this study it is not explained whether the tested antibody preparations were absolutely comparable. Both Stephan and Trautmann established minor differences in the survival rate of the individual groups, while Ax et al. found no differences. Nevertheless, all authors agreed that unsplit immunoglobulin and $F(ab')_2$ molecules provided protection, whereas Fab fragments did not. The effectiveness of both unsplit immunoglobulin and pure $F(ab')_2$ preparations (prepared each time from the same hyperimmune serum) could be established during the treatment (post infection) of distemper virus infections in ferrets, rabies infections in guinea-pigs and foot-and-mouth disease virus infections in baby mice. In those control groups with no antibody administration the various infections proved fatal for the animals (Johannsen et al. 1982; Johannsen et al., in preparation). One can conclude, on the basis of these data, that $F(ab')_2$ molecules in vivo possess an antibacterial and antiviral effect comparable to that of unsplit immunoglobulin.

These results are supported by a series of experiments testing the effect of a $F(ab')_2$ preparation, administered either alone or combined with various antibiotics (including Cefotaxim), on the death rate of mice infected with various bacteria including human pathogenic bacteria (Staphylococcus aureus 108, Streptococcus Aronson B, Pneumococcus mucosus, Escherichia coli 978, Salmonella typhimurium, Pasteurella multocida etc. (Ronneberger and Zwisler 1977; Klesel and Limbert 1980; Ishibashi et al. 1980). In these experiments a clear antibacterial property of $F(ab')_2$ preparations and/or a synergistic effect with antibiotics could be demonstrated. These findings are supported by in vitro experiments on germ inhibition (Zwisler and Ronneberger 1978). During the course of these studies it was possible to show, in vitro, that the production of ampicillin-resistant mutants of Staphylococcus areus was significantly inhibited by the administration of $F(ab')_2$ preparations of IgG (Zwisler and Joachim 1978).

Despite this effectiveness in pharmacological models, the relevance to the clinical situation is still questionable. The problems involved can be illustrated by the herpes infection. Carter and Easty (1981) reported clear effects following the administration of antibodies (unsplit) in the case of experimental ulcerative herpes ceratitis in rabbits, as long as the treatment was begun before, at the time of, or shortly after the onset of the infection. Oakes and Lausch (1981) were able to establish a similar effectiveness in the case of

herpes simplex infection in mice. $F(ab')_2$ preparations proved as effective as unsplit immunoglobulins but not, however, when used following the infection. Here $F(ab')_2$ fragments were not as effective as unsplit immunoglobulin, and were even ineffective. In contrast, clinical studies have demonstrated the effectiveness of $F(ab')_2$ preparations both in herpes-induced cerato uveitis (Moriyama and Manabe 1979) and in herpes zoster (Okita et al. 1979).

The results of clinical studies on the antibacterial and antiviral effectiveness of immunoglobulin preparations have mainly been published as case reports. Ewald (1979) provided a survey of the casuistic contributions. Such case reports can only provide indications as to the effectiveness of $F(ab')_2$ antibodies (in terms of figures, mainly therapeutically) or of unsplit immunoglobulins (mainly prophylactically). There is a lack of random prospective and controlled informative clinical studies with the help of which the clinical effectiveness would be soundly and statistically proven. There is also a lack of a double blind study, above all because no suitable placebo has as yet been available. This information gap cannot (or only to a limited extent) be closed by retrospectively controlled and successful studies, such as those of Neu (1980) on infections of the central nervous system. An available two-armed random prospective controlled study on the therapy of sepsis and bacterial pneumonia has not been able to demonstrate a therapeutical advantage of $F(ab')_2$ preparations. This was mainly due to an optimal antibiotic therapy by means of which the mortality rate in the control group could be reduced from 38 to nought for septicemia and from 44 to one for pneumonia (Lindquist et al. 1981). Under these optimal conditions it is, of course, very difficult to show the statistical significance of the effect of an additional treatment with a gammaglobulin preparation.

A further prospective randomized controlled study shows that the $F(ab')_2$ preparation, administered within 48 h of rectum extirpation (10 g, split into three doses) caused a statistically significant reduction of the infection rate and an improvement in wound healing (rate of primary healing) which thus shortened the length of the hospital stay (Probst and Fabian 1979). Another controlled study on selected cases of abdomen and thorax operations also established that the postoperative administration of unsplit immunoglobulin (β-propiolacton-treated, 20 g per patient) statistically significantly reduced the number of local infections and the rate of septic complications (Duswald et al. 1980). Thus these two clinical studies point, despite all differences, in dosage for example, to the basically comparable antibacterial effect of $F(ab')_2$ preparations and intact immunoglobulin.

Besides their antibacterial and antiviral effectiveness, antibodies can neutralize toxins. This activity, however, seems to be limited to exotoxins, since as yet there have been few indications that the biological toxic activity of endotoxins can be inhibited by antibodies (Urbaschek 1975 and 1976). The role of antibodies against endotoxins, which can always be found in polyvalent immunoglobulin preparations, thus remains unclear.

Exotoxins can be neutralized by specific antibodies. In this respect unsplit antibodies, $F(ab')_2$ and Fab molecules appear to be of the same value. Experimental and clinical experience has been obtained with $F(ab')_2$ preparations, produced from heterologous antibodies against exotoxins (fermosera), which have been preferred to unsplit heterologous immunoglobulins for same time on account of their better tolerance (Weil et al. 1938; Schmidt 1939; Arlt 1940).

Recently arguments have been put forward for the better effectiveness of $F(ab')_2$ and Fab fragments in the speedy elimination of low molecular antigens. Thus, in the case of digitalis poisoning, Butler et al. (1977) and Ochs et al. (1978), using Fab fragments in dogs, and Hess et al. (1979),, using $F(ab')_2$ preparations in humans, demonstrated this rapid therapeutical effect which was superior to that of intact immunoglobulin (Ochs et al. 1978) and which they attributed to the faster tissue penetration and the shorter half-life of the split products and to the increased excretion through the kidneys (Butler et al. 1977) of the immune complexes which had accumulated in the serum. These properties of $F(ab')_2$ preparations are possibly responsible for the success of Eckert et al. (1980) in dealing with experimental peritonitis in rats and peritonitis in humans.

Activation of Complement

The comparable antibacterial and antiviral effectiveness of $F(ab')_2$ molecules and unsplit immunoglobulin in vivo should also be demonstrable in appropriate in vitro test methods. An essential question in this respect is whether $F(ab')_2$ molecules are able to activate complement. In the past this was denied, possibly for methodological reasons (Spiegelberg and Götze 1972). Today there are a number of studies from various groups which agree that IgG-$F(ab')_2$ antibodies are able to activate complement via the alternative but not via

the classic pathway (Amiraian and Leikhim 1961; Schur and Becker 1963; Isliker et al. 1967; Gadd and Reid 1981; Juarez et al. 1981; Albar et al. 1981) and thus are able to lyse normal cells (Johannsen et al. 1977), virus infected cells (Ehrnst 1977; Perrin et al. 1976) or tumour cells (Jackson and Grant 1976). Using the Vibrio cholerae model, Steele and Rowley (1977) and Steele et al. (1977) could show that $F(ab')_2$ antigen complexes demonstrate a reduced complement activation and opsonization in vitro but that the bactericidal activity and the protective efficacy of $F(ab')_2$ molecules are fully preserved in vivo. Due to the recent results of work done by Johannsen et al. (1980), showing that unsplit immunoglobulins can activate the alternative complement system in the same way as $F(ab')_2$ antibodies, a possible explanation for the preserved antibacterial activity of $F(ab')_2$ preparations in vivo would be that in vivo the complement activation involved in the elimination of bacteria and viruses occurs largely via the alternative pathway, or at least that the two pathways are equally effective. There are indeed indications that the alternative pathway plays the dominating role in vivo (Takahashi et al. 1980). Thus it is known that bacterial infections often appear in cases of hereditary C3 deficiency, but less frequently in cases of homozygous deficiency of those complement factors C1r, C4 or C2 (survey: Fearon and Austen 1980) involved in complement activation via the classic pathway.

Studies on cytolysis of culture cells infected with measles virus (Sissons et al. 1979), herpes simplex type-1 virus, influenza A virus or mumps virus (Perrin et al. 1976) demonstrated that unsplit IgG and $F(ab')_2$ were equally effective. This indicates that cytolysis was probably mediated predominantly via the alternative pathway. Fab fragments were much less effective or not effective at all. Here Sissons et al. (1979) contradicted Ehrnst's (1977 and 1978) thesis that antibodies against measles fusion protein only act cytolytically via the classical pathway of complement activation. Moreover Sissons et al. (1979) and Perrin et al. (1976) showed quite clearly by using human cells, human antibodies and their fragments and human complement that, in humans too, $F(ab')_2$ molecules activate complement via the alternative pathway.

Phagocytosis and Exocytosis

The comparable antibacterial and antiviral effect of $F(ab')_2$ molecules and unsplit immunoglobulin in vivo should not only be reflected in the activation of complement, but should also be detectable in phagocytosis models in vitro. In order to satisfy this condition, only those phagocytosis models that are most stringent to the in vivo situation should be chosen. The work of Mannik and Arend (1971), Mannik et al. (1971) and Haakenstad and Mannik (1977) has demonstrated that antigen-antibody complexes are predominantly taken up by macrophages in vivo and not, or to a lesser extent, by granulocytes. Moreover, experiments with mouse peritoneal macrophages and immune complexes, produced from radioactively labelled tetanus toxoid and human antibodies against tetanus toxoid (Schorlemmer et al. 1981) have shown that $F(ab')_2$ immune complexes, like IgG immune complexes, are absorbed by macrophages. Since these results contradict to some extent earlier statements (Berken and Benacerraf 1966), according to which the opsonizing activity of antibodies is dependent on the integrity of the Fc part and on the binding affinity of the Fc part to the Fc receptor of the phagocytozing cell, the mechanism of the opsonizing activity of $F(ab')_2$ molecules on macrophages was more closely examined. Tests established that $F(ab')_2$ immune complexes activate macrophages to release lysosomal enzymes by means of the activation of complement – in particular C3 (Schorlemmer et al. 1980). Since C3 itself is synthesized by macrophages, no additional complement appears to be required for this mechanism (Schorlemmer et al. 1976 and 1977). The addition of complement, especially of C3b, can however increase phagocytosis and the intracellular degradation of antigen-antibody complexes to a high degree, as has been demonstrated both in vitro and in vivo (van Snick and Masson 1978).

The binding of antigen-antibody complexes to and phagocytosis by macrophages can thus take place to a similar extent either through Fc receptors and/or through complement receptors. It therefore does not seem to be of decisive importance whether or not the immune complexes contain unsplit IgG or $F(ab')_2$) molecules; what is important is how many Fc and complement receptors the macrophage carries. It seems worth mentioning here that the number of Fc and complement receptors increases after the activation of macrophages which should logically lead to an increase in their phagocytosing ability (Bianco et al. 1975). Moreover, the opsonizing activity of antibody preparations (of unsplit immunoglobulins, too) appears to be significantly dependent on the ability to activate complement, especially C3. For instance Fab molecules or reduced and alkylated antibodies are either no longer able to activate complement at all or their complement activating capacity is re-

duced. Parallel to this, the ability of these antibody preparations to eliminate antigens in vivo, that is to say, to make them susceptible to phagocytosis, is also drastically reduced (Mannik and Arend 1971; Haakenstad and Mannik 1976; van Snick et al. 1978; Ax et al. 1981).

An additional argument for the subordinate role of the Fc receptors during the in vivo elimination of antigen-antibody complexes (Mannik and Arend 1971; Mannik et al. 1971; Haakenstand and Mannik 1977) might be the following fact: antibodies of the IgM class are generally regarded as optimal antibacterial agents; human macrophages and granulocytes, however, possess few or no Fc receptors for IgM, respectively (survey: Theophilopoulos and Dixon 1980). On the other hand, immune complexes with antibodies of the IgG class are much more phagocytosed by macrophages after incubation with an IgM rheumatoid factor (van Snick et al. 1978; Onyewotu et al. 1975) and much less so by granulocytes (Messner et al. 1968). These findings point to the fundamental role of complement activation and complement receptors in phagocytosis of antigen-antibody complexes by macrophages (Shavit et al. 1979). Indications have been given by the work of Menzel et al. (1978) and especially by that of Horwitz (1980) that this mechanism could also apply to granulocytes and their dominating role in the phagocytosis of bacteria. Horwitz, in particular, provided evidence for the significance of antibodies and complement in phagocytosis and the killing of bacteria by neutrophil granulocytes and macrophages. Using encapsulated and non-encapsulated E. coli he was able to show that granulocytes are far superior to macrophages in this respect and that complement is imperative for phagocytosis and the killing of bacteria. No specific antibody is required for optimal phagocytosis and killing of bacteria directly able to activate C3, whereas the phagocytosis and killing of bacteria such as encapsulated E. coli, which cannot activate C3, take place only in the presence of a specific antibody and complement, but not with antibody alone. Thus, according to the model system employed by Horwitz (1980), the fundamental function of the specific antibody during the phagocytosis of bacteria by granulocytes and macrophages is the local activation of complement, in particular of C3.

Besides the fundamental task of neutrophile granulocytes – early phagocytosis, mostly of particular substances – another important function of these cells is the mediation and intensification of inflammatory reactions by release of lysosomal enzymes (e.g. acid and/or neutral hydrolases, plasminogen activators, elastase and cathepsins; survey: Ignarro 1974; Ryan 1977) and of lysosomal cationic proteins capable of degranulating human basophile granulocytes and mast cells. The release of lysosomal substances activates the Hagemann factor and systems dependent on this (kinin system, coagulation system and fibrinolysis system) and the complement system, all of which intensify inflammation together with mediators from macrophages, mast cells, blood basophils and thrombocytes. The exocytosis of the contents of lysosomes is associated with phagocytosis in macrophages and granulocytes but, as the example of the so-called "frustrated" phagocytosis (Henson 1971) in granulocytes shows, it is not necessarily dependent on it. Experiments by Hofstaetter et al. (1981) have shown that exocytosis of enzymes from human granulocytes by antigen-antibody complexes, produced with tetanus toxoid and $F(ab')_2$ fragments of a human antibody, is only induced in the presence of complement, and this to a much lesser extent than by comparable immune complexes with unsplit antibody.

The results obtained by Horwitz (1980) are generally confirmed by comparative studies carried out by Leijh et al. (1981) on the phagocytosis and killing of Staphylococcus aureus and E. coli by neutrophile granulocytes. However, in contrast to IgG, no opsonizing effect of either IgM or $F(ab')_2$ fragments of IgG could be demonstrated. Tympner et al. (1976) showed that yeast cells opsonized with $F(ab')_2$ antibodies are less well phagocytosed by granulocytes in vitro than those treated with unsplit antibodies. Similar results were obtained by Stendahl et al. (1977) in in vivo and in vitro phagocytosis experiments with dead bacteria previously treated with antibody preparations. Eibl and Friers (1978) even found a competitive effect in vitro of $F(ab')_2$ and Fab molecules on the phagocytosis of E. coli bacteria by granulocytes induced by antiserum. These results have not been reproduced as yet. Moreover, there is as yet no correlation in relevant animal models; on the contrary, the positive results of experimental in vivo studies with living germs contradict these in vitro findings. Furthermore, studies on healthy test persons have shown that the bactericidal activity of the peripheral granulocytes distinctly increases after i.v. injection of $F(ab')_2$ preparations (Lindquist et al. 1978).

In addition to the interaction of antigen-bound $F(ab')_2$ molecules or unsplit antibodies with Fc or complement receptors of macrophages and granulocytes, the interaction with thrombocytes is also of significance. Human thrombocytes possess

Fc receptors for IgG to which antigen-antibody complexes or immunoglobulin aggregates can bind (Henson and Spiegelberg 1973; Müller-Eckhard and Lüscher 1968; Ohtsu et al. 1979). Such interaction leads to the aggregation of thrombocytes and to the release of vasoactive amines such as serotonin, adrenalin, histamine, ADP and various clotting factors. This binding and release does not take place with immune complexes containing $F(ab')_2$ molecules. Pretreatment of the thrombocytes with Fc fragments completely inhibits the immune-complex-dependent exocytosis (Isreaels et al. 1973; Weisbarth et al. 1979). The mediators released by the thrombocytes intensify the course of the inflammation. It is of significance here that serotonin released by thrombocytes during interaction with immune complexes drastically increases the toxic effect of endotoxin (Urbaschek 1975 and 1976). Thrombocytes are also stimulated to exocytosis by lysosomal substances from granulocytes and macrophages as well as by a number of other factors, such as complement and clotting factors (survey: Sedlacek 1980b). Since the release of lysosomal substances from granulocytes after interaction with $F(ab')_2$ immune complexes is also considerably reduced in comparison to IgG immune complexes, a reduced release of vasoactive substances from thrombocytes induced by this mechanism can also be expected.

This theory is supported by experiments in which shock reactions were induced in mice by means of i.v. injections of tetanus toxoid-IgG immune complexes. However, no shock reactions were observed after the injection of comparable $F(ab')_2$ immune complexes. Moreover, neither did the expected shock reaction occur when $F(ab')_2$ fragments of an antibody directed against the Fc part of IgG in the immune complex were injected immediately after the administration of IgG immune complexes (Sedlacek et al. 1979a and b).

Discussion of Table 2 and Summary

Each administration of immunoglobulins to an organism has the aim of eliminating certain antigens, either infectious organisms or toxins, by means of antibody binding. The antigen-antibody complexes or immune complexes which are formed trigger cellular and humoral reaction chains which serve to kill the infectious agents or remove the antigen. The immune complexes can, however, also cause illnesses – so-called immune complex diseases (survey: Haakenstad and Mannik 1977; Sedlacek 1980a and b) – by means of these mechanisms. In this context mention must be made of the side-effects, the "phlogistic reactions" published by Schnoz (1979), which appeared in a relatively high percentage of agammaglobulinemic patients after substitution with an unsplit antibody preparation and which were attributed to the in vivo formation of antigen-antibody complexes.

The cellular and humoral reactions on which these side-effects are based, take place in healthy humans every day in the course of active cellular and humoral immune resistance to ubiquitous infectious organisms. They are mainly a result of the interaction of antigen-bound antibodies, via their Fc parts, with Fc receptors of granulocytes, thrombocytes and macrophages. In vivo and in vitro data show that exocytosis of mediators and lysosomal substances, such as enzymes, basic peptides and vasoactive substances is a major outcome of these interactions. These substances increase the penetrability of vessels and thus lead to an increased infiltration of the relevant tissue area by cells and blood proteins. The activation, caused either directly or indirectly by immune complexes, of humoral systems such as complement system, Hagemann factor and systems dependent on these (coagulation system, fibrinolysis and kinin system) augments the penetrability of vessels. These reactions help in the resistance to infectious organisms. In this capacity they were developed in nature as sensible mechanisms. They are regulated by inhibitors and inactivators and by proteases. These counter-regulation mechanisms normally prevent clinical symptoms.

The overtaxing of these counter regulatory mechanisms can lead to clinical symptoms which can be assigned to immune-complex illnesses. The risk of such an overtaxing may arise when specific antibodies are administered to an infected organism and many immune complexes arise within a short period of time. One example of this is the classic shock reactions seen in animals after infusion of antigen followed by antibodies, or after i.v. injection of immune complexes (Sedlacek et al. 1979a). The risk of an immune complex illness in the clinic must be kept as small as possible by the responsible physician. This means that he should be familiar with the special properties of the various antibody preparations and should use this knowledge when selecting immunoglobulin preparations for the various clinical indications.

It was shown above that in the course of an inflammatory reaction an organism is capable of splitting IgG into $F(ab')_2$ and Fab molecules by the release of proteases. These fragments both retain their antigen-binding properties: the $F(ab')_2$ fragment is bivalent, that is to say it binds two

Table 2. Properties of IgG and IgG split products

	IgG	$F(ab')_2$	Fab
Natural occurrence	++	+	+
Half-life time (blood)	~18 days	2 days	<1 day
Increase of catabolic rate for IgG	++	−	−
Antigen-specific immunosuppression	++	−	−
Complement activation:			
– classic	++	−	−
– alternative	++	++	−
Toxins:			
– neutralisation	++	++	++
– elimination	++	++	++
Hapten-elimination	++	+++	+++
Antibacterial effect (in vivo)	++	++	+/−
Antiviral effect (in vitro/in vivo)	++	++	+/−
Phagocytosis			
Macrophages			
via Fc-receptors	++	−	−
via C3b-receptors	++	++	−
Granulocytes			
via Fc-receptors	++	−	−
via C3b-receptors	++	+	−
Exocytosis:			
Macrophages			
via Fc-receptors	++	−	−
via C3b-receptors	++	++	−
Granulocytes			
via Fc-receptors	++	−	−
via C3b-receptors	++	+	−
Exocytosis and aggregation:			
thrombocytes	++	−	−

antigen determinants like unsplit IgG, whereas the Fab fragment is monovalent. There are clear differences in their biological properties. These are summarized in Table 2. They are evident in half-life time in the blood, in influence on IgG catabolism and in antigen-specific – and possibly also antigen-unspecific – immune suppression. Furthermore, in contrast to Fab fragments, $F(ab')_2$ molecules are both antibacterially and antivirally effective as IgG; Fab fragments are also equally effective as $F(ab')_2$ molecules and unsplit IgG in their ability to neutralize toxins, whereas Fab and $F(ab')_2$ preparations are able to eliminate haptens more quickly than unsplit IgG. There are differences in complement activation, too: in contrast to immune complexes, the antibody share of which is unsplit IgG, immune complexes containing $F(ab')_2$ do not activate the classical pathway but only the alternative pathway of complement. Immune complexes from Fab fragments do not significantly activate either pathway.

Also, there are differences in the interaction of antigen-bound unsplit or fragmented immunoglobulins with phagocytes and with thrombocytes. Thus, the release of lysosomal enzymes and inflammation mediators from granulocytes after contact with immune complexes with $F(ab')_2$ molecules is reduced, when compared to immune complexes with unsplit IgG. Both types of immune complexes are taken up by macrophages and induce the release of enzymes. In contrast to immune complexes with IgG, there is no direct interaction with thrombocytes by immune complexes with $F(ab')_2$ antibodies, that is to say, there is neither platelet aggregation nor release of the various thrombocyte mediators including serotonin. Serotonin considerably increases the toxic effect of endotoxin. What conclusions for the clinic can be drawn on the basis of these differences between IgG and its $F(ab')_2$ and Fab split products? With regard to the half-life time it is clear that for the prophylactic administration of immunoglobulins to healthy persons, and for substitution of immunglobulins in cases of hereditary or acquired hypo- or agammaglobulinemia, a long presence of the preparation in the blood is desirable in order to reduce the frequency of the treatment to a minimum. There is no doubt as to the experimental and clinical effectiveness of immunoglobulin preparations in prophylaxis (Morell and Barandun 1982). The risks involved in this treatment concern problems of compatibility but not the secondary reactions which can be triggered by a massive antigen-antibody complex reaction. However, the latter must be feared in all apparent and inapparent infections and poisonings after the administration of antibody preparations, especially in acute therapeutical use.

Here, as already mentioned above, there is unfortunately a considerable lack of statistically instructive, relevant, controlled, clinical studies showing the effectiveness of immunoglobulins. This deficit becomes even greater with regard to comparative studies of different immunoglobulins, for instance unsplit IgG versus $F(ab')_2$ preparations. However, it is only by means of such studies that an answer can be found to the question as to whether or not the experimental findings on immune suppression by immune complexes containing IgG in patients with no impairment of their immune system are clinically significant; the same applies as to whether or not the better compatibility of $F(ab')_2$ preparations, due to the lack of interaction with Fc receptors on thrombocytes and granulocytes, in humans – as in animal experiments – represents an advantage over unsplit IgG in acute therapy of contagious diseases and cases of poisoning.

On the other hand, irrespective of such clinical comparative studies, it is safe to conclude that $F(ab')_2$ and Fab preparations should be superior

to unsplit immunoglobulin preparations in the elimination of toxins, especially those of low molecular weight, due to the increased ability of the respective immune complexes to pass through the kidneys. Moreover it is conceivable that while the short half-life time of $F(ab')_2$ preparations is a disadvantage in prophylactic treatment and substitution over longer periods, it could be regarded as an advantage in the therapeutical situation.

Thus, in the case of infection, specific antibodies are administered to fight the usually unknown infectious organisms by means of an injection of a polyvalent antibody preparation with the aim of substituting the lack of these very specific antibodies, and thus to achieve a better elimination of the pathogen. It is clear that the passively introduced specific antibodies are used up within a few hours so that, depending on the course of the illness, a repetition of the administration at short intervals often becomes necessary. When unsplit immunoglobulin preparations with long half-life times are used, this repeated administration over a short space of time leads to an increase of the serum level of antibodies which are irrelevant for the pathogen in question. As far as we know at present this also leads to an increase in the catabolic rate of the administered and newly synthesized immunoglobulins.

In contrast, when preparations of immunoglobulin split products, such as $F(ab')_2$, are used, the antibodies in the preparations which are not acutely required are eliminated or catabolised in a relatively short time. Furthermore there are no indications that the catabolism of the body's own IgG antibodies in the recipient is increased after administration of $F(ab')_2$ or Fab antibodies. Thus a frequent treatment with $F(ab')_2$ preparations in higher doses ought to be less problematic. This thesis, however, has also not yet been proved by sound data from comparative clinical studies. Such studies are therefore necessary in order to find out whether the possible advantage of an immunoglobulin preparation that has been demonstrated in experimental tests is also demonstrable in defined clinical indications and especially in questions of therapy.

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