Agents and Actions
vol. 6 · 1/2/3 (1976)
Birkhäuser Verlag, Basel

Biological Roles of Rodent Anaphylactic IgG_1 Antibodies

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Abstract

Described and mainly studied in guinea-pigs and mice (Benacerraf, Ovary, Bloch and Franklin, 1963; Fahey, Wunderlich and Mishell, 1964) (but also demonstrated in rats and hamsters, and others), IgG_1 immunoglobulins are a fascinating class of antibodies. Long known for their anaphylactic properties, they have now been also studied in our laboratory for their cooperative, protective and immunoregulatory properties. Like other immunoglobulin classes of antibody, their biological activity is exerted through their Fc portion and expressed only after the immunoglobulin has been activated by fixation on corresponding antigen through their Fab portions.

My purpose is to present a summary of the work done in our laboratory on the biological role of IgG_1 antibodies.

This presentation will be divided into 3 parts respectively concerned with their inflammatory properties in specific hypersensitivity reactions such as anaphylaxis and Arthus, their protective action (as a final result) of the antigen-bearing target in the facilitation reaction, and finally the relations (and common denominator) between the two types of properties.

Results and discussion

(1) Inflammatory properties of IgG_1 antibodies in specific hypersensitivity reactions

These have been studied and are well-defined in anaphylaxis with soluble (usually protein) antigens.

(A) *Studies on Arthus reactions*

In guinea-pig Arthus reactions, it has been described (Ovary, Benacerraf and Bloch, 1963) that IgG_1 antibodies are responsible for increased vascular permeability (IVP) and oedema while IgG_2 are independently responsible for hemorrhage and polymorphonuclear (PMN) attraction through C′ fixation. Actually, the role of IgG_1 in Arthus reactions is more important than previously described as we have shown (Maillard and Voisin, 1970), since (a) their action is necessary in order to obtain an efficient hemorrhagic action through IgG_2 antibodies

Table 1
Characteristics of arthus reactions when antigen injected 48 hours after γ_1, γ_2 antibodies or a combination of both[1])

Antibodies injected	Amount of precipitating antibody in circulation		Antibody titers in sera at 48 hours		Arthus reactions		
	At time of injection	at 48 hours (assumed)[2])	Passive Hemagglutination	Passive Hemolysis	Edema (μl)	V.P.I. (μg of dye)	Hemorrhage (mm²)
γ_1	1,3 mg[3])	0,52 mg	128, 128[4])	0, 0	354, 426	23, 60	0, 0
γ_2	2,6 mg	0,52 mg	128, 256	128, 128	35, 105	2, 13	7, 3
$\gamma_1 + \gamma_2$	1,08 mg (γ_1) + 0,52 mg (γ_2)	0,53 mg ($\gamma_1 + \gamma_2$)	128, 128	12, 12	461, 346	47, 44	201, 78

[1]) The time is chosen beyond the phase of high rate elimination of the isolated antibodies.
[2]) Assuming a same rate of elimination for precipitins and hemagglutinins.
[3]) mg of antibody protein.
[4]) Individual figures for the 2 guinea-pigs of a group.
(From Maillard and Voisin, Proc. Soc. exp. Biol. Med. *133,* 1188, 1970).

(Table 1) and (b) they are able to induce, by themselves, not only important IVP and oedema but also a conspicuous attraction of PMN. This chemotactic action has also been evidenced and studied in vitro (J. Leung-Tack, F. Beucher, Maillard and Voisin, 1974) where it was shown to be at least as intense as the one of IgG_2 immune complexes, not to depend on C'_1 activation but on C'_3 activation (alternate pathway) and on the contact system since kaolin and DFP which inhibit it without modifying the complement system decrease the chemotaxis induced by IgG_1 (but not IgG_2) immune complexes (Fig. 1). It has also been shown that the IgG_1 induced Arthus reaction depends on the concentration of IgG_1 antibodies contained in the tissues rather than the one in the blood and is independant from blood complement as well as histamine (Maillard and Voisin, 1973). This type of IgG_1-induced inflammation has been termed 'white Arthus'.

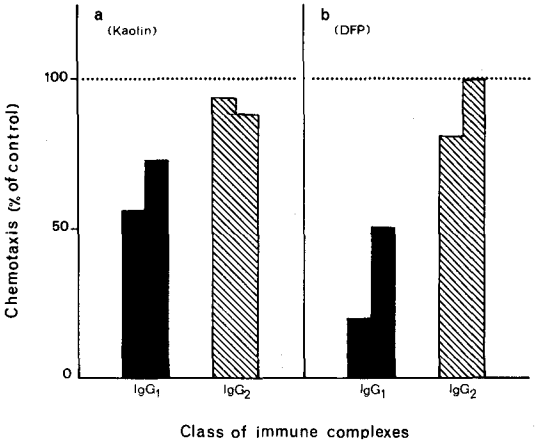

Figure 1
Effect of kaolin and DFP treatment of fresh serum upon the chemotactic response to IgG_1 and IgG_2 complexes.
In (a) fresh serum was absorbed on kaolin (5 mg per ml, 15 minutes at room temperature) and centrifuged before addition of complexes. In (b) DFP, 10^{-3} *M*, is present during incubation with complexes, then, after a centrifugation, dialyzed out. Chemotaxis is figured as the percentage of the maximal chemotaxis, given by each appropriate control, i.e. the different doses and classes of complexes reacting upon untreated fresh serum. For each group of 2 bars shown, the first one represents the response to 250 μg and the second to 2,500 μg of complexes with fresh serum treated either by kaolin or by DFP; all control values are 100% by definition.
(From J. Leung-Tack, F. Beucher, Maillard and Voisin, Int. Arch. Allergy *47*, 609-622, 1974.)

(B) *Studies on auto-immune lesions*

An autoimmune aspermatogenic orchiepididymitis (AIAO) can be induced in guinea-pigs by at least 3 independant spermatozoa autoantigens termed S, P and T (Voisin and F. Toullet, 1968). The disease can be passively transferred by immune sera directed against P and T antigens, not S - against which only anaphylactic IgG_1 antibodies are produced - (F. Toullet and Voisin, 1974). It has recently been shown that IgG_2 are responsible for the passive transfer by anti-T sera while IgG_1 are necessary for the passive transfer by anti-P sera. It had previously been demonstrated that P gives rises to IgG_2 C'-fixing, precipitating antibodies and to IgG_1 anaphylactic antibodies. It is also responsible for strong hemorrhagic Arthus reactions. Whether IgG_1 play the same type of cooperative role with IgG_2 in AIAO passive transfer, as it does in passive Arthus, remains to be elucidated; at least is it suggested.

(C) *Studies on allotransplantation systems*

The question arose whether allotransplantation antigen-antibody systems were able to induce hypersensitivity reactions of the classical types. Studies were conducted leading to the new concept of 'transplantation anaphylaxis'. This concept is based on three types of demonstration.

(1) Transplantation immune sera contain

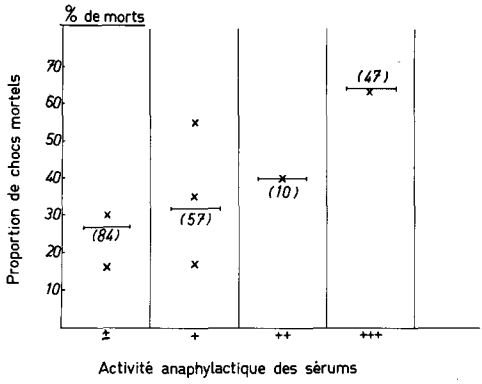

Figure 2
Correlation of mortality attributable to the immunoallogeneic shock with the anaphylactic activities of CBA anti-A sera.
X = individual experiments; — = mean of experiments.
No of animals is indicated in parentheses.
(From J. Voisin, Kinsky and Voisin, Transplantation *15*, 206-210, 1973.)

anaphylactic antibodies, evidenced by PCA tests, where intravenous antigen is made of soluble H-2 preparations and intradermal immune sera are injected in normal mice syngeneic to the sera. These antibodies are IgG_1 in hyperimmune sera where they have been tested (Kinsky, Voisin, Hilgert and Duc, 1971).

(2) Transplantation immune sera result in a severe, often lethal shock, when injected i.v. into mice against H-2 specificities for which they are directed. This shock may be attributed to a passive reverse anaphylaxis for the following reasons: the aspect and chronology of the shock are similar to those of classical anaphylaxis; it is completely inhibited by anti-histamine, anti-serotonin (Phénergan); it is consistently correlated with the anaphylactic properties of the sera utilized (Fig. 2), or their chromatographic fractions, and not with their C′-fixing activity. This phenomenon has been termed immuno-allogeneic shock (Voisin, Kinsky and Voisin, 1973). Like PCA, it is not evident in all strain combinations while the following phenomenon has been found to take place in every H-2 incompatible tested strain combination.

(3) Mast cells are known to bear at their surface Fc receptors for anaphylactic antibodies, the activation of which leads to active degranulation. They also bear transplantation antigens. It was therefore conceivable that allogeneic transplantation anaphylactic antibodies could bind themselves to mast cell transplantation antigens through their Fab specific binding sites and to the Fc receptors of the very same mast cells through their Fc reactive portion, thus triggering the degranulation process. It was indeed found to occur, and the phenomenon

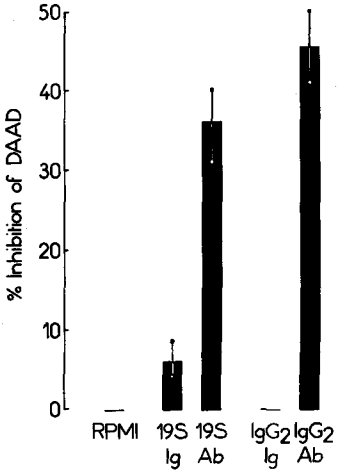

Figure 4
Inhibitory effect of mast cells preincubation with BALB/C anti EL4 19S and IgG_2 specific antibodies (Ab) on DAAD elicited with a fraction giving a standard (75%) degranulation. Normal BALB/C immunoglobulins (Ig) obtained in identical conditions did not inhibit significantly DAAD. (From Daëron, Duc, Kanellopoulos, Le Bouteiller, Kinsky and Voisin, Cell. Immun., in press 1975.)

has been called direct allogeneic anaphylactic degranulation (DAAD). Mast cells are specifically and immediately degranulated when incubated with an alloantiserum directed against histocompatibility antigens borne by the mast cells (Fig. 3). The behaviour of the mast cells is identical to that observed during classical anaphylaxis with respect to microscopic and ultrastructural morphology and kinetics and different from mast cell immunotoxic lysis by C′ and C′-fixing antibodies. The phenomenon is immunologically specific and the responsible antigens were shown to be of the classical so-

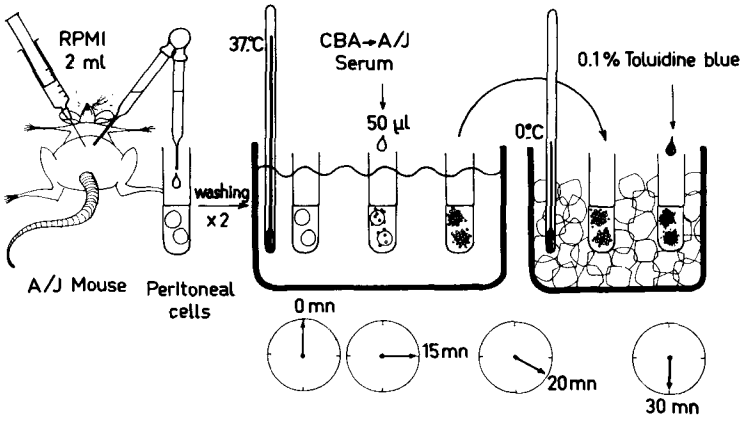

Figure 3
Sequence of operations in the DAAD test.
(From Daëron, Duc, Kanellopoulos, Le Bouteiller, Kinsky and Voisin, Cell. Immun., in press 1975.)

called 'SD' type and not to be IA antigens or linked to specific receptors; neither were they allotypic immunoglobulin determinants. The phenomenon is complement-independant but needs the integrity of the Fc portion of the antibodies. Neither 19 S nor IgG_2 antibodies are efficient; however they are able to inhibit the reaction through Fab competition for antigen (Fig. 4). IgG_1 can be implicated as the responsible antibodies. Heat-labile anaphylactic antibodies with characteristics of IgE (but not identified as such) has some activity in early transplantation immune sera. They are soon overwhelmed by IgG_1 and seem to disappear (Fig. 5) (Daëron, Duc, Kanellopoulos, Le Bouteiller, Kinsky and Voisin, 1975).

Therefore, together with the description of the PCA directed against transplantation antigens and that of the immunoallogeneic shock, DAAD contributes to the concept of transplantation anaphylaxis, the biological meaning of which should be determined.

(2) Protective action of IgG_1 antibodies on antigen-bearing target (facilitation reaction)

While inflammatory properties of IgG_1 antibodies in specific hypersensitivity reactions have been discovered with conventional soluble protein antigens and only recently applied to auto-antigens and transplantation antigens, on the opposite side, the protective and regulatory activity of this antibody class was first discovered and analyzed in the field of transplantation and tissue or cell antigens and only recently applied to chemically-defined conventional antigens.

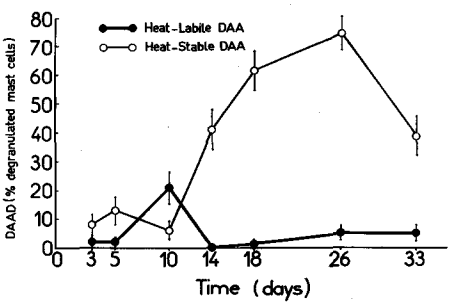

Figure 5
Evolution of heat-stable and heat-labile DAAD responses in the serum of CBA mice immunized by a single injection of Sa I cells.
The appearance of heat labile activity is restricted to day 10 after immunization.
(From Daëron, Duc, Kanellopoulos, Le Bouteiller, Kinsky and Voisin, Cell. Immun., in press 1975.)

(A) *Studies on allotransplantation systems*

As a first in vitro model of peripheral action, the competition experiments during which anti-sheep red blood cell (SRBC) IgG_1 antibodies could protect SRBC against lysis induced by C′ and C′-fixing IgG_2 must be mentioned (Kourilsky, Bloch, Benacerraf and Ovary, 1963). This required a 50 to 1 ratio for efficient protection.

More fundamental is the Immune Facilitation Reaction concept derived from the enhancement phenomenon. The latter was the phenomenon by which an allotransplanted tumor is enhanced in its acceptance and growth by virtue of antibodies directed against the tumor and actively produced or passively introduced in the host. The former (facilitation reaction) is 'the mechanism (s) by which an antibody or an immune reaction (immune cells) promotes the persistence of the corresponding antigen and the integrity of the cytological structures which support it, by preventing it from inducing or undergoing (or both) immune rejection' (Voisin, 1971). The early demonstration that a similar phenomenon may prevent a graft-versus-host reaction (GVHR) (Voisin and Kinsky, 1962) opened the way to the following situations: normal tissue allografts, autoimmune diseases, autochtonous tumor evolution and even immune reactions in pregnancy. All these situations were eventually found to be highly relevant to the concept of the facilitation reaction.

One of the most important problems is to identify the responsible immune agents. They seem to be of two kinds: cells of the so-called suppressive type that are very much studied but not well defined yet and antibodies that have been termed enhancing, facilitating or, in some in vitro situations, blocking. What is their nature? Unlike several groups of American workers, we have localized in IgG_1 anaphylactic antibodies the bulk of the facilitating-enhancing activity. In our pioneer studies (Voisin, Kinsky and Jansen, 1966) as well as in subsequent studies (Voisin, Kinsky, Jansen and Bernard, 1969), physical fractionation of immune transplantation sera by curtain or Pevikon block electrophoresis or DEAE cellulose chromatography consistently showed the bulk of enhancing activity in IgG_1-containing fractions while

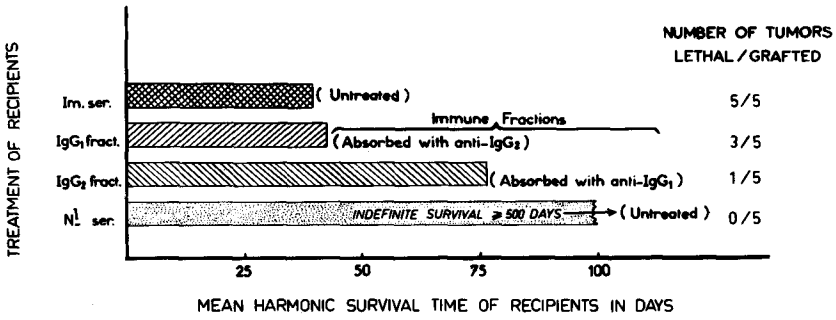

Figure 6
Enhancing activities of CBA anti-A/Jax antibody classes. Fractions (from a DEAE cellulose column, eluted at 0.05-0.07 NaCl equivalent) containing both IgG_1 and IgG_2 in comparable concentration were pooled and thoroughly immunoabsorbed with either anti-IgG_2 or anti-IgG_1 monospecific sera. The test system consisted in allografting Sa I on CBA mice after fraction (or serum) injection and appreciating the number of lethal takes in each group as well as the mean survival time of the recipient mice.

(Modified from Duc, Kinsky, Kanellopoulos and Voisin, J. Immun., in press 1975.)

cytotoxic IgG_2 antibodies were inhibitory, especially at high dosage. More recently (Duc, Kinsky, Kanellopoulos and Voisin, 1975) immunoabsorption experiments with monospecific anti-IgG_2 and IgG_1 immune sera lead to the same conclusions (Fig. 6). Another approach was to correlate the facilitating activity with other biological properties caused by antibodies of known Ig classes (such as C′ fixation and anaphylaxis). This was carried out either for transplantation immune whole sera obtained by several procedures or for DEAE fractions of given transplantation immune sera. In both cases, the link was with anaphylactic activity,

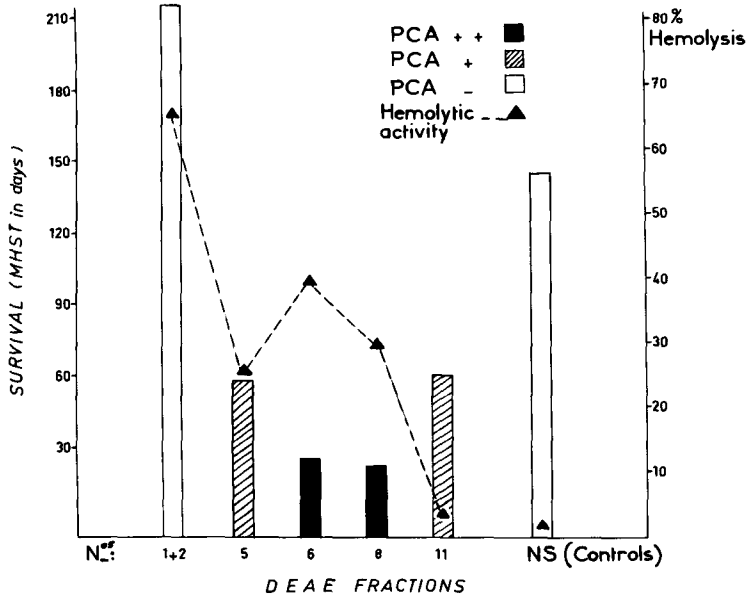

Figure 7
Association of PCA and enhancement-facilitation activities in DEAE immune serum fractions.
Enhancing power of fractions is expressed by MHST in days of CBA male recipients grafted with Sa I cells. The strongest enhancement is expressed by the shortest survival time. The same number of hemagglutination units (50) were injected by volume adjustments. NS = normal CBA serum.
(From Kinsky, Voisin and Duc, Transplantation *13*, 452–460, 1972.)

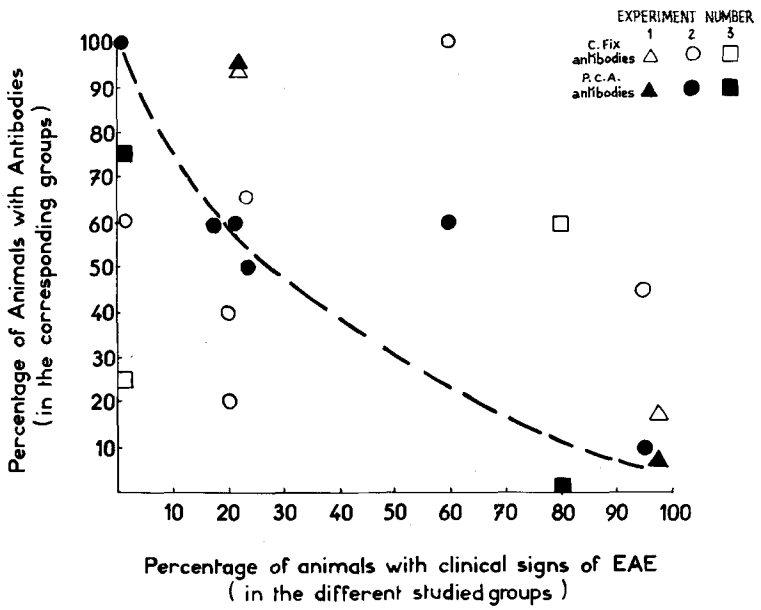

Figure 8
Inverse relationship between the incidence of anaphylactic antibodies and the incidence of the disease in different experimental groups.
CF antibodies: △, ○, □ (experiments 1, 2, 3, respectively). PCA antibodies: ▲, ●, ■ (experiments 1, 2, 3 respectively).
(From Lebar and Voisin, Int. Arch. Allergy *46*, 82–103, 1974.)

while C′-fixing, cytotoxic activity was rather linked to in vivo inhibitory properties (Fig. 7). Recently however, it was found that dilution and immune complexing could confer facilitating properties to IgG_2 antibodies (Duc et al. 1975).

(B) *Studies on auto-immune lesions*

These protective, facilitating properties of IgG_1 antibodies are not limited to immune transplantation sera and allograft phenomena. In auto-immune situations, there are suggestions that IgG_1 antibodies may be endowed with protective properties: immune sera from guinea-pigs protected against AIAO (by testis antigens mixed with incomplete adjuvants) contained PCA (but no cytotoxic) antibodies against testis extracts; they were furthermore able to prevent the in vitro spermotoxic action of C′-fixing antibodies found in the sera of animals presenting the lesions (after treatment with testis antigens mixed with complete adjuvants) (Chutna and Rychlikova, 1964a, 1964b). Along a similar line, a correlation was found between the percentage of protection against experimental allergic encephalomyelitis (EAE) in guinea-pigs and the incidence of anaphylactic antibodies (identified as IgG_1) (Lebar and Voisin, 1974, and Fig. 8).

Another potentially important phenomenon has been discovered during pregnancy in the mouse. Maternal immunoglobulins are present on the placenta, at the surface of the trophoblastic cells. Part of these immunoglobulins cannot be washed out but only eluted by acidic buffer. There are at least partly anti-paternal antibodies, mainly of the IgG_1 class. When injected into other mice of the same strain as the mother, they inhibit the capacity of these mice to reject a tumor of the father's strain (Table 2).

The reconciliation of this presumed protective activity of IgG_1 antibodies in auto-immune situations with their apparently cooperative activity with IgG_2 antibodies in lesion induction remains to be elucidated.

(C) *Studies with defined conventional non-cell-membrane antigens*

Table 2
Facilitating-enhancing action of placental eluates on Sa I (A/Jax) tumors grafted on C57 Ks mice**.*

Origin of placental eluates		Number of grafted mice	Lethal takes	Rejections
father	mother			
A/Jax	C57 Ks	10	9	1
C57 Ks	C57 Ks	15	0	15

* 2×10^6 Sa I grafted cells on either male or female C57 Ks.
** Males or females.

Not only the preceding conclusions can apply to non-cell-membrane, conventional defined antigens, but also these may enable us to understand the underlying mechanisms. In active facilitation reactions, tissue antigens administered in a certain way (soluble, intravenous, in high dosage) will prevent the classical challenge (such as skin graft or experimentally induced auto-immune disease) to reach the stage of immune rejection, while sensitized cells and IgM and IgG_2 C′-fixing cytotoxic antibodies (the two types of immune rejection agents) are depressed and anaphylactic IgG_1 antibodies (and possibly suppressive cells) are not, or even sometimes increased. This phenomenon has its counterpart with soluble, non cell-borne antigens (such a bovine serum-albumin for instance) in the phenomenon known as immune deviation. The only difference is that there is no antigen-bearing target, either cell or tissue in the latter.

It was interesting to see whether IgG_1 that were not decreased (and sometimes increased) during the phenomenon played a role in it. Purified IgG_1 (and IgG_2) anti-DNP (or anti-carrier) antibodies were injected into guinea-pigs together with DNP-BGG and their immune response was followed in terms of IgG_1 and IgG_2 anti-DNP antibodies. It was eventually found that passively administered IgG_1 and IgG_2 were about equally suppressive on the active formation of anti-DNP IgG_2 antibodies. However, concerning the active formation of anti-DNP IgG_1 antibodies, passive anti-DNP IgG_2 was suppressive but IgG_1 was not; it was only delaying the response, but increased it after a while, especially after a booster. Meanwhile, it was of interest that passive anti-carrier IgG_1 antibody was most suppressive (Vuagnat, T. Neveu and Voisin, 1973a, 1973b, and Fig. 9).

This type of experiment is far from being contradicted by Turk and Parker's results (1972) since these authors found that cyclophosphamide injected 3 days before immunization suppressed antibody formation (and apparently mainly IgG_1) preferentially and resulted in increased delayed hypersensitivity of the Jone's-Motte type. Whatever the role played by suppressive cells in the various preceding phenomena, IgG_1 antibodies - especially in the form of immune complexes - seem to play a regulatory role characterized by preferential inhibitions precisely of the immune agents of the rejection reaction. This is precisely the homeostatic and protective role of the facilitation reaction.

(3) Relations (and common mechanisms?) between the two types of properties

In a very general way this chapter might amount to a straight-forward question: what is the biological meaning of anaphylaxis? Without pretending to answer the question, the problem of common mechanisms is being approached from two sides.

(A) *The role of mast cell degranulation*

Anaphylactic reactions such as they are induced by IgG_1 and IgE antibodies following contact with corresponding antigens are triggered by the release from activated mast cells of active substances such as histamine and serotonin. Does this mast cell degranulation process and the released substances play a role in immunoregulation? This could also be in keeping with the existence of histamine receptors at the surface of certain lymphocytes, the removal

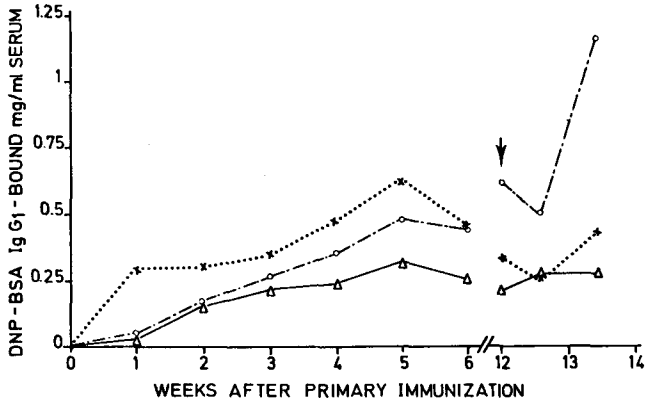

Figure 9
Effect of passive IgG_1 antibodies (to hapten or to carrier) on the IgG_1 anti-hapten response. Groups of six guinea-pigs were immunized on day 0 with 25 μg of DNP_{51}-BGG either alone (X...X) or mixed with 500 μg of purified anti-HGG IgG_1 (△——△) or anti-DNP IgG_1 (0–.–.0) antibodies. A booster injection of 100 μg of antigen in saline was given on the 12th week (arrow).
(From Vuagnat, T. Neveu and Voisin, J. exp. Med. *137*, 265–274, 1973.)

of which leads to an increased immune response (Shearer, Melmon, Weinstein and Sela, 1972).

The primary in vitro immune response was studied according to Mishell and Dutton's technique. The intensity of the response as judged by the number and the proportion of plaque-forming cells per culture, was found to be decreased by 50 to 70% by the following preparations: histamine or serotonin, or both, as well as a supernatant of mast cells degranulated by a specific reaction (unrelated to SRBC) (Fig. 10). Also efficient was the addition to the culture of mast cells passively sensitized to SRBC. An inhibition of this suppressive activity, that is the return to a normal or subnormal level of PFC, was obtained when antihistamines were utilized. Suggestions for an in vivo activity of these supernatants of degranulation were recently obtained (H. Fallah, Maillard and Voisin, 1975).

(B) *The role of the plasmin system*

Isolated IgG_1 are able (as are IgG_2) to activate plasmin and even to firmly bind it but only after contact with the specific antigen (Maillard and C. Favreau, 1975). This suggests a mechanism reminiscent of that of C′ activation and fixation. Does this process play a role in immune regulation? This is currently studied in our laboratory by J. Maillard in the Mishell and Dutton's system. Preliminary results favor this possibility.

A new possibility arises therefore: that the very same cellular mechanisms that lead to hypersensitive inflammation and anaphylactic shock may be at work in the homeostatic process of immune regulation.

A last point pertains to the observed differences in the behaviour of transplantation immune sera utilized in vitro and in vivo. The very same serum may be cytotoxic in vitro for the corresponding target - in the presence of complement - while it may enhance the in vivo acceptance of the same target cells. This discrepancy might be understood on the assumption that cytotoxic IgG_2 and IgM antibodies and facilitating IgG_1 antibodies are both present in these sera (and this is usually so) and that IgG_1 extravasate at a faster rate than IgM, IgG_2 (and possibly C′). This possibility would favor the protection of the target and the initiation of a facilitation reaction. The normal vascular permeability of purified IgG_1, IgG_2 and of IgM was studied using a paired radiolabelled technique and it was found indeed that IgG extravasate much faster than IgM, and IgG_1 faster than IgG_2 (D. Jullien-Vitoux and Voisin, 1973, and Fig. 11).

Among many unanswered questions, a few very puzzling seem of particular interest: What are the functional relations (and differences) between IgG_1 and IgE, and what is the human equivalent for IgG_1? What is the survival advantage of IgG_1 (and IgE)-induced increased vascular permeability and smooth muscle contraction? Last but not least, what are the functional relations between anaphylactic antibodies or immune complexes and suppressive or regulatory cells?

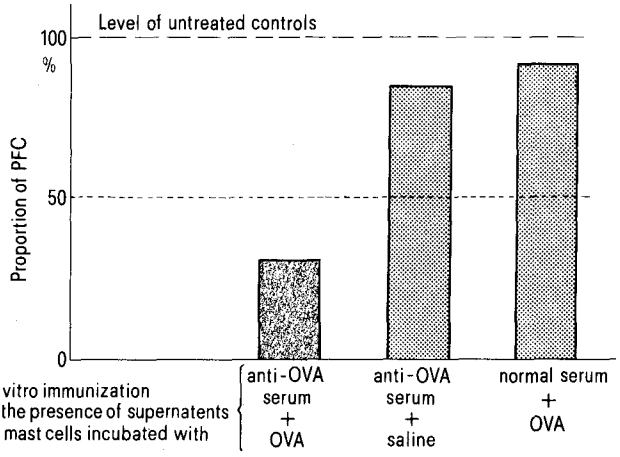

Figure 10
Depressive action of supernatants from specifically degranulated mast cells on the primary immune response in vitro.
Mast cells are degranulated by an ovalbumin/anti-ovalbumin reaction. The released substances depress the in vitro immune response against sheep red blood cells.
(From H. Fallah, Maillard and Voisin, 1975.)

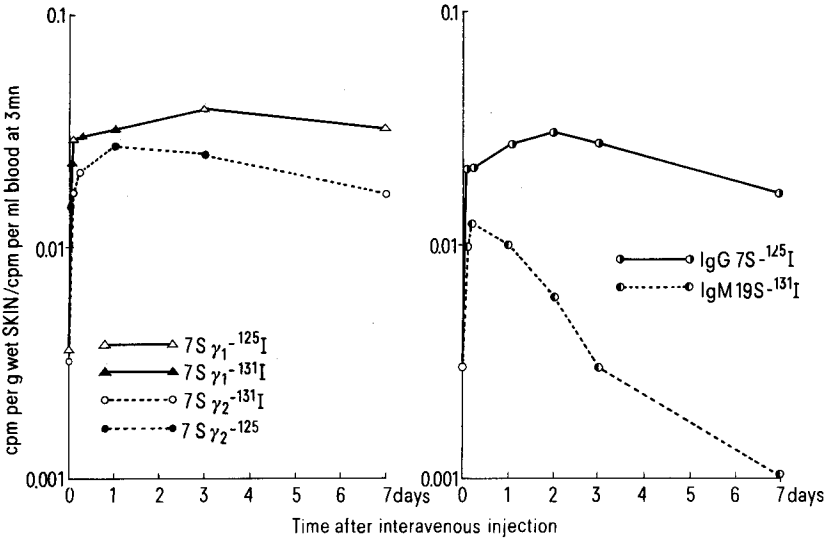

Figure 11
Kinetics of 7S, 19S, 7Sγ_1 and 7Sγ_2 immunoglobulins extravasation in normal guinea-pig skin. (From D. Jullien-Vitoux and Voisin, Eur. J. Immun. *3*, 663–667, 1973.)

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Discussion

Turk (UK)

Thank you very much, Dr. Voisin. You were quite up to your usual standard and thank you for a very masterly talk. This is a most important subject that Dr. Voisin has raised – the role of IgG_1, as opposed to IgG_2, in the phenomenon that is generally known as immunological enhancement. Many other workers feel that the immunological antibody in immunological enhancement phenomenon is IgG_2, but over the years Dr. Voisin has given us much convincing evidence of the role of IgG_1.

Voisin (France)

May I answer your comment on the role of IgG_1 and IgG_2 in the enhancement phenomenon, and more broadly in the whole reaction, which is opposed to simply the graft rejection phenomenon. In recent experiments with Kinsky and Duc we have once again confirmed the constant role of IgG_1 but we have been able to induce an enhancing role in IgG_2 by dilution of the IgG_2; they become enhancing when they are diluted. Secondly, by complexing them with the appropriate antigen, and then also they become enhancing. There is some recent work by Rubinstein and his co-workers, showing that only IgG_1 is enhancing at any dose, while IgG_2 and IgM are suppressing at high doses, whils enhancing at low doses.

Turk (UK)

Are there any other questions? Well in that case I would like to change the topic with another question. Could you perhaps go into a little more depth about the work that Jean Maillard and you did some time ago on the role of IgG_1 in the Arthus reaction? Jean talked about this in Yugoslavia once, and you referred to him directly in your talk here. How do you feel about the two Arthus reactions? The one induced by IgG_1 and the one induced by IgG_2 differ. And do you feel that complement plays a role? I think I will leave it at that for the moment, and perhaps we can develop it further.

Voisin (France)

I did refer to this topic rather rapidly. However, when Ovary and co-workers used the reversed Arthus reaction in order to ascertain the respective roles of IgG_1 and IgG_2, these provoke local inflammation that may produce hemorrhage when there should not be hemorrhage. We have used with Jean Maillard general passive Arthus, where we do absolutely no harm and sometimes one can even inject the antigen 30 minutes before the antibody intravenously. Therefore there is no damage locally, and when doing that there is IgG_2 which is unable to induce even hemorrhage, by itself. The cooperation of IgG_1 that increases vascular permeability and tissue permeability seems to be necessary, and, by itself, IgG_1 is able to attract and to give a picture that looks like an Arthus reaction, but without any hemorrhage but with many polymorphonuclears. This chemotaxis, as studied in vitro, is due to two phenomena. By suppressing the activity of C'_1 and keeping C'_3 one does not suppress the chemotaxis, but there is a decrease by inhibiting the contact system. I suppose Jean Maillard may be in the audience and he may want to contribute something. No he is not here.

Turk (UK)

We have been very interested in this phenomenon of yours. We of course are great supporters of you in the IgG_1 battle and in our system, where we immunized guinea-pigs with 1 μg of ovalbumin in Freund's incomplete adjuvant, we skin tested them with 100 μg of antigen at 14 days. Under these conditions they produce

a pure Arthus reaction and the only antibody detectable in their serum is an IgG_1. We can produce a local passive transfer with the serum of these animals (i.e. a reverse passive transfer) which does not have a hemorrhagic component, although the actively sensitized animals do. The Arthus reaction is not affected by treatment of the actively-immunized or passively-immunized animals with cobra-venom factor.

Voisin (France)

After the work that we did with Jean Maillard, which was stimulated by the results of the work with Francine Dulard with this autoantigen S which gives rise to other pure IgG_1 antibodies, this autoantigen S, when injected into the skin of immunized animals, gives a big Arthus without hemorrhage. The same type, but this is not anaphylaxis. It develops progressively and is maximal after several hours and with a polymorphonuclear attraction.

Turk (UK)

At last we have attracted some response from the audience.

Wilkinson (UK)

I really felt that I must break into this dialogue. I just wanted to remind you whilst talking about the chemotactic activity of IgG_1 for polymorphs, of some work Ralph Snyderman did some years ago, when he looked at the activity of both IgG_1 and IgG_2 and he looked at this in both normal and C'_4-deficient guinea-pigs. Basically what he found was that in the C'_4-deficient guinea-pigs, IgG_1 would activate chemotactic activity, presumably through the alternate pathway, but the IgG_2 would not. So that it looked like IgG_1 will activate chemotaxis through the alternate complement pathway. I don't know whether you agree with that?

Voisin (France)

It seems to work by two pathways that are not by direct complement activation. That is, C'_3-activation because when one suppresses C'_1, IgG_2 has no more chemotactic activity, but IgG_1 still has. But when one suppresses C'_3, there is a decrease in IgG_1 chemotactic activity, but there is something left. There is also a decrease by the drugs that are known to interfere with the contact system, and known not to act with the complement system. All this was verified as you understand in the work which has been done over many years, and I have only presented a summary here.

Turk (UK)

Has anyone else any data on the role of complement in Arthus reactions?

Willoughby (UK, France)

Yes, we have some recent data on the role of the complement system in the Arthus reaction, but as this forms a rather substantial part of the presentation that I will be giving on Saturday I prefer to wait until then. I trust that those of the audience who survive the three days until Saturday will be able to hear those aspects of this work discussed at that point in time.