Response to autologous immunoglobulin G in patients with rheumatoid arthritis

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It has been suggested that the chronic inflammation characteristic of rheumatoid arthritis (RA) represents an immunopathological response to an exogenous or endogenous constituent present in the joint (see review by Glynn, 1968). Dumonde and Glynn (1962) have speculated that sensitization to a product of inflammation could initiate a self-perpetuating process, and in support of this hypothesis have produced experimental chronic arthritis in the rabbit by the injection of fibrin into the knee joints of animals previously immunized with that protein. The experiments of Hollander and his associates have suggested that intra-articular reaction to autologous immunoglobulin G (IgG) may provoke inflammatory effusions in RA patients but not in normal subjects (Restifo, Lussier, Rawson, Rockey, and Hollander, 1965). There was a degree of specificity in these reactions, in that IgG from other subjects was only active when the donor himself had RA and the IgG possessed at least two Gm groups in common with the autologous globulin (Hollander, Fudenberg, Rawson, Abelson, and Torralba, 1966).

In view of these considerations, it was decided to investigate the inflammatory response of RA patients to intradermal injection of autologous aggregated IgG. This material readily binds anti-globulin (rheumatoid) factors and should demonstrate whether reaction with these antibodies leads to prolonged inflammatory changes comparable to those occurring in the joint but in a more readily observable site. Furthermore, aggregated γ-globulin, like immune complexes, gives rise to an acute inflammatory response. There is an initial erythematous reaction clearly visible by 30 minutes, which depends upon fixation of the globulin to skin and activation of complement (Christian, 1960; Ishizaka and Ishizaka, 1964). The generation of chemotactic activity through fixation of complement components causes an influx of polymorphonuclear leucocytes (Ward, Cochrane, and Müller-Eberhard, 1966) associated with oedema and erythema reaching a maximum within a few hours. Induction of this ‘Arthus-type’ reaction thus provides an opportunity for testing the hypothesis of sensitization to a product of inflammation.

Material and methods

Patients
Eleven patients with definite or classical rheumatoid arthritis (9 female and 2 male) were studied. The mean age was 52-7 years (range 28 to 73). All but two were sero-positive. One patient had atopic eczema and sensitivity to a wide variety of allergens. Two were receiving systemic prednisolone therapy (6 and 8 mg. daily respectively). Fifteen controls were studied, of whom ten were female. The mean age was 44-8 years (range 20 to 76). Two had osteoarthritis of the hips and one had generalized osteoarthritis; the remaining twelve volunteers were healthy members of staff. None suffered from allergies and none was receiving steroids.

To examine the influence of anti-inflammatory drugs on the response to intradermal γ-globulin, a group of nine patients suffering from degenerative joint disease was examined; they were receiving soluble aspirin, phenylbutazone, indomethacin, or prednisolone in doses comparable to those commonly used in the treatment of rheumatoid arthritis.

Human γ-globulin preparations
To avoid possible transmission of serum hepatitis, all glass apparatus was treated with chromic acid; other apparatus and materials were used only once. IgG fractions were prepared from fresh samples of serum by chromatography on DEAE-cellulose. Approximately 8 to 9 ml. of sera were equilibrated with 0-02M phosphate buffer pH 6-4 and run onto a 35 x 1-5 cm. column of DEAE-cellulose (Whatman, DE 52). The breakthrough peak was concentrated in a dialysis sac immersed in solid sucrose, then dialysed against 0-85 per cent. pyrogen-free saline. The preparations were sterilised by filtration through Millipore membranes and routinely checked for bacterial contamination. Preparations of pooled γ-globulin prepared by ether precipitation (Kekwick and Mackay, 1954) (kindly supplied by Dr. W. d’A. Maycock) were reconstituted and sterilized similarly.
The concentration of γ-globulin was assessed by measurement of optical density, using the relationship

\[ E = 0.5 \text{ cm. } 0.1 = 139 \mu g./\text{ml.} \]

and levels were adjusted to approx. 20 mg./ml. Aliquots were aggregated by heating to 63°C for 10 min. in a water bath. Where indicated complement-fixation was carried out using a scaled down version of the method described in Kabat and Mayer (1961).

**DERMAL RESPONSE TO γ-GLOBULIN**

In the experiments comparing arthritic patients and controls, each subject was injected intradermally at widely separated sites on the forearms and occasionally on the upper arms, with 0.2 ml. of each of three different γ-globulin preparations: viz., native autologous IgG, aggregated autologous IgG, and aggregated pooled γ-globulin. The preparations were randomly distributed between these sites in different subjects. The arms were examined under a 100 watt blue light at 30 min. and at 1 hr, and then hourly for the next 7 hrs. Measurements of the diameter of erythema and swelling were recorded. Where the responses were asymmetric, two diameters at right angles were measured and the geometric mean used for analysis.

**Results**

**Intradermal response to γ-globulin in normal subjects**

Immediately after injection of native autologous IgG (NA-IgG), an area of erythema developed around the injection site, but in most cases this had resolved by 10 minutes and between 30 and 60 minutes the skin appeared normal. In the majority of subjects a secondary reaction involving erythema and oedema was observed reaching a maximum at 3 to 8 hours and subsiding thereafter. Examination at 24 hours revealed no superficial abnormality.

The immediate erythematous reaction to aggregated autologous IgG (AA-IgG) was significantly more marked than with native IgG (Table I) and was observed invariably, tending to reach a maximum between 30 and 60 minutes. All of the individuals studied also showed a secondary reaction, maximal between 3 and 8 hours, which was characterized by erythema and, in all but two cases, substantial oedema. In some subjects the two responses merged and were less clearly defined (Fig. 1). The secondary reaction was always greater than that given by NA-IgG (Tables II and III, opposite).

![Figure 1](image_url)  
*Fig. 1 Intradermal response to autologous and homologous heat-aggregated IgG. The individual IgG preparations were heat-aggregated and adjusted to give the same degree of complement-fixation per unit volume before injection. The diameter of the swelling (and erythema) produced followed a biphasic course and no difference between the responses to autologous and homologous IgG was evident.*

Reactions produced by injection of aggregated pooled γ-globulin (APG) were similar but were significantly less extensive at 3 to 8 hours than those observed with AA-IgG (Tables II and III).

The degree of variation of the 3 to 8 hour response was assessed by making multiple injections of a constant dose of AA-IgG into different sites on the arms. In one individual receiving eight injections, the coefficient of variation was 17 per cent., and in another injected at six sites, a coefficient of 24 per cent. was obtained.

It was found that maximum 3 to 8 hour reactions were obtained with AA-IgG using approximately 3 mg. protein. The standard dose subsequently employed was 2 mg.

**Table I**  
*Dermal erythema 30-60 min. after injection of γ-globulin into rheumatoid arthritis patients and controls*  
(Mean diameter (mm.) ± SE)

<table>
<thead>
<tr>
<th>Subjects</th>
<th>No. tested</th>
<th>Autologous IgG</th>
<th>Aggregated IgG</th>
<th>Aggregated pooled γ-globulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA</td>
<td>11</td>
<td><em>14.8 ± 4.7</em></td>
<td>31.6 ± 3.0</td>
<td>28.5 ± 3.0</td>
</tr>
<tr>
<td>Controls</td>
<td>15</td>
<td><em>1.1 ± 0.9</em></td>
<td>28.8 ± 2.1</td>
<td>29.3 ± 2.1</td>
</tr>
</tbody>
</table>

*Significantly different from aggregated autologous IgG injected into same group; P < 0.05; NSD = No significant difference
Table II  Dermal erythema 3-8 hrs after injection of γ-globulin into rheumatoid arthritis patients and controls  
(Mean diameter (mm.) ± SE)

<table>
<thead>
<tr>
<th>Subjects</th>
<th>No. tested</th>
<th>Autologous IgG Native</th>
<th>Aggregated</th>
<th>Aggregated pooled γ-globulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA</td>
<td>11</td>
<td>*15.9 ± 1.4</td>
<td>25.6 ± 2.8</td>
<td>*14.3 ± 3.3</td>
</tr>
<tr>
<td>Controls</td>
<td>15</td>
<td>*17.1 ± 1.8</td>
<td>26.9 ± 2.5</td>
<td>*11.5 ± 1.4</td>
</tr>
</tbody>
</table>

*Significantly different from aggregated autologous IgG injected into same group: P < 0.05

Table III  Dermal swelling 3-8 hrs after injection of γ-globulin into rheumatoid arthritis patients and controls  
(Mean diameter (mm.) ± SE)

<table>
<thead>
<tr>
<th>Subjects</th>
<th>No. tested</th>
<th>Autologous IgG Native</th>
<th>Aggregated</th>
<th>Aggregated pooled γ-globulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA</td>
<td>11</td>
<td>*10.7 ± 2.7</td>
<td>24.5 ± 5.0</td>
<td>*10.5 ± 3.3</td>
</tr>
<tr>
<td>Controls</td>
<td>15</td>
<td>*9.6 ± 2.2</td>
<td>33.3 ± 4.4</td>
<td>*13.0 ± 2.9</td>
</tr>
</tbody>
</table>

*Significantly different from aggregated autologous IgG injected into same group: P < 0.05

Intradermal response to γ-globulin in patients with rheumatoid arthritis

The responses of rheumatoid patients to NA-IgG, AA-IgG and APG were not significantly different from those obtained in the normals (Tables II and III; Figs 2 and 3) except for the erythema produced at 30 to 60 minutes by NA-IgG. This was only seen in one of fifteen normal subjects compared with six out of eleven patients. Again the late erythema and swelling caused by the APG was significantly less than that induced by AA-IgG.

A group of patients without inflammatory joint disease who were receiving anti-inflammatory drugs in doses of the same order as those taken by the RA patients did not differ in their responses to intradermal γ-globulin. Thus heat aggregated autologous IgG gave an erythematos reaction of 35 ± 4 mm. (mean diam. ± SE) at 30 to 60 minutes and 26 ± 4 mm. at 3 to 8 hrs, and a swelling of mean diameter 38 ± 7 mm. at the later time.

Comparison of individual immunoglobulin-G preparations

The greater effectiveness of autologous IgG as compared with γ-globulin from a large pool of individual sera suggested that a degree of specificity may exist. This possibility was examined by comparing the intradermal response to AA-IgG and to other preparations of IgG which had been isolated...
individually from the sera of four different recent blood donors. After heat aggregation, each preparation was tested for its ability to bind complement. The concentrations were then adjusted so that all preparations fixed the same amount of complement per unit volume of 0.2 ml. of each IgG was injected intradermally in triplicate into the back of a normal subject. There was no apparent difference between the mean diameter of the erythema caused by autologous IgG and that evoked by each of the four homologous globulins (Fig. 1).

Discussion

Intradermal injection of autologous heat-aggregated IgG into human subjects produced an immediate erythema followed by an 'Arthus-type' reaction involving erythema and oedema with a maximum at 3 to 8 hours. This declined thereafter and by 24 hours only minimal reactions could be observed. There was clearly no delayed type hypersensitivity component involved.

The similarity in the 3 to 8 hour response to aggregated autologous IgG in RA patients and normals suggests that antiglobulin factors do not play any significant role in producing chronic inflammatory lesions and that sensitization to a product of acute inflammation does not appear to be implicated in rheumatoid disease. These considerations presuppose that the skin provides a valid test site. If however locally synthesized avid antibodies capable of causing tissue damage were absorbed out by antigen in the joint, only the weakly avid antibodies would reach the circulation and these could well be incapable of generating a skin reaction on injection of the aggregated IgG. Furthermore, the antiglobulins which react with heat aggregated IgG are probably not complement-fixing; whether the same is true of antiglobulins directed against other determinants such as those on the Fab portion exposed by immune complex formation has yet to be established. The possibility that treatment with anti-inflammatory drugs was masking an intrinsically higher response in the RA patients was discounted by the finding that a group of patients without inflammatory joint disease receiving these drugs in comparable dosage showed a similar range of skin reactivity to the controls.

Reactions to aggregated pooled γ-globulin were also comparable in patients and controls. However, within each group, these were generally smaller than the reactions to autologous protein. Further study showed that the difference was probably related to a reduction in the degree of aggregation during heating due to the presence of contaminating serum constituents in the pooled globulin rather than to any specificity of the individual IgG molecules which would have implied involvement of antiglobulin factors.

The only major difference between the RA patients and controls was seen in the 30-minute reaction to native autologous IgG. The significance of this reactivity in the rheumatoid arthritis is at present obscure. The preparations from rheumatoid patients may have contained appreciable concentrations of aggregated IgG or possibly the histamine releasing factor (?) antigen-antibody complexes) described by Baumal and Broder (1968). Perhaps the kinin-forming activity of IgG is potentiated in rheumatoid sera. It is also possible that these sera contain tissue autoantibodies which could release pharmacologically active agents through combination with antigen.

Summary

Autologous heat-aggregated IgG injected intradermally into human subjects produced an immediate erythema followed by an 'Arthus-type' reaction. Responses in patients with rheumatoid arthritis were no greater than in normal subjects suggesting that sensitization to a product of inflammation is not implicated in rheumatoid disease. Also, in so far as these results can be extrapolated to events occurring in the joint, they provide no support for the view that antiglobulin factors play a major role in producing chronic inflammation. The lower responses obtained with pooled γ-globulin could be ascribed to the presence of contaminating serum proteins which reduced the degree of aggregation on heating before injection. No delayed reactions were observed.

A response at 30 minutes to native autologous IgG was observed in six out of eleven rheumatoid patients but in only one out of fifteen controls. Possible reasons for this are discussed.

We wish to thank Dr. A. C. Boyle for allowing us to carry out these studies on patients under his care. We acknowledge the assistance of Dr. G. Torrigiani, Sister G. Rogers, Mr. H. S. Drury, and Mrs. G. Stead. The work was supported in part by grants from the Arthritis and Rheumatism Council and the Medical Research Council.
References


ISHIZAKA, K., AND ISHIZAKA, T. (1964) J. Immunol., 93, 59 (Molecular basis of passive sensitization. II. The role of fragment III of γ-globulin and its disulfide bonds in passive sensitization and complement fixation).


Résumé

L’effet de l’immunoglobuline G autologue chez les malades atteints d’arthrite rhumatoïde

L’immunoglobuline G autologue agrégée par la chaleur et injectée par voie sous-cutanée chez l’homme a produit un érythème immédiat suivi d’une réaction du type Arthus. L’effet chez les malades atteints d’arthrite rhumatoïde n’était pas plus marqué que chez les sujets sains, suggérant ainsi que la sensibilisation à un produit inflammatoire n’est pas impliqué dans la maladie rhumatoïde. Aussi, dans la mesure où ces résultats peuvent être extrapolés aux changements qui ont lieu dans l’articulation, ils ne donnent pas l’impression que les antiglobulines jouent un rôle prépondérant dans la production de l’inflammation chronique. Les effets moins marqués obtenus avec un mélange de γ-globuline peuvent être attribués à la présence de la contamination par des protéines sériques qui ont diminué le degré d’agréagation par la chaleur avant l’injection. Aucune réaction secondaire n’a été observée.

Un effet 30 minutes après l’injection de l’immunoglobuline G autologue et innée a été observé chez six des onze malades, mais chez seulement un des quinze témoins. Les raisons probables de cette observation sont discutées.

SUMARIO

Respuesta a la inmunoglobulina autóloga en pacientes con poliartritis reumatoide

La inyección extraférica de IgG autóloga con agregado de calor aplicada a sujetos humanos produjo eritema inmediatamente, seguido de una reacción tipo Arthus. Las reacciones en pacientes con poliartritis reumatoide no fueron mayores que en sujetos normales, lo cual sugiere que la sensibilización a un producto de inflamación no se presenta en la enfermedad reumatoide. Asimismo, hasta donde estos resultados pueden ser extrapolados con fenómenos que ocurren en la articulación, estos resultados no apoyan el concepto de que los factores antigulobulinos desempeñan un papel importante en cuanto a producir inflamación crónica. Las respuestas bajas obtenidas con una combinación de globulina y podrían ser atribuidas a proteínas contaminadoras del suero, que redujeron el grado de agregación de calor antes de la inyección. No se observaron reacciones tardías.

Se observó una reacción a los treinta minutos de haber inyectado IgG autóloga natural en seis de once pacientes, pero solamente en uno de quince testigos. Se discuten las posibles razones de este fenómeno.