THE CONGLUTINATION PHENOMENON

XI. IMMUNO-CONGLUTININ IN HUMAN SERA

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(With 4 Figures in the Text)

Immuno-conglutinin may be defined as an immune substance produced in the serum of an animal following an antigenic stimulation and which has the property of reacting with complement adsorbed on an antigen-antibody complex or other surface causing a marked clumping or flocculation of the reactants *in vitro*. Immuno-conglutinin derives its name from its similarity in activity to the naturally occurring conglutinin found in the serum of healthy ruminants. It has been suggested (Coombs & Coombs, 1953) that immuno-conglutinin and conglutinin have a definite although as yet undefined role in immunity.

In previous papers (Coombs & Coombs, 1953; Coombs, 1954) it was found that conglutinin or immuno-conglutinin may be present in small amounts in the sera of normal rabbits, rats and mice and in larger amounts in the sera of normal guinea-pigs. In guinea-pigs the level varied between a serum titre of 8 and 128. No conglutinin has been demonstrated in horse's serum.

In rabbits, rats and mice immuno-conglutinin could be induced in the serum to a high titre by the intravenous injections of Salmonella organisms, Proteus OX 19 and other unrelated organisms. The immuno-conglutinin is in no way related in specificity to the injected antigen. The implication is that the immuno-conglutinin response is directed against the animal's own complement, which in the adsorbed state is able to act auto-antigenically, and the process has been referred to as one of auto-stimulation.

This paper extends these investigations to man. Human sera from apparently healthy persons and from persons with certain diseases have been examined for their content of immuno-conglutinin.

MATERIALS AND METHODS

Estimation of immuno-conglutinin

The immuno-conglutinin level of serum was determined by Method II*b* given in paper IX of this series (Coombs & Coombs, 1953).

The sera to be tested had their complement inactivated by heating at 56° C. for 30 min. and were absorbed with washed sheep red cells (10 drops packed cells to 1 ml. serum) to remove sheep-cell antibodies.

Serial doubling dilutions of the sera were prepared in 0·1 ml. volumes. To each dilution were added 0·1 ml. saline and 0·1 ml. of a 0·4% suspension of alexinated sheep cells (see below). The mixtures were incubated for 30 min. in the 37° C.

water-bath. The degree of clumping was recorded macroscopically after centrifuging the tubes and resuspending the deposited cells in the original supernatant fluid. The end-point or conglutinin titre was the serum dilution which gave a clear background with discrete visible clumps.

Each day's estimations were controlled by including a human and a rabbit serum having previously determined immuno-conglutinin titres.

Preparation of a suspension of alexinated sheep cells

Sheep red cells were sensitized with bovine antibody by incubating at 37° C. for 15 min. 2·5 ml. of a 4% suspension of sheep cells with 2·5 ml. of heat-inactivated bovine serum diluted 1/2 in saline. The cells were deposited by centrifugation, washed once in saline and saline added to 2·5 ml. to reconstitute a 4% suspension.

To alexinate the sensitized cells a mixture was made up of the following:

2·5 ml. 4% suspension of sensitized cells.

2·5 ml. undiluted horse serum as complement.

2·5 ml. undiluted heat-inactivated horse serum added to ensure ample component 4 of complement.

17·5 ml. saline.

After incubation at 37° C. for 20 min. the cells were deposited by centrifugation, washed once in saline, and finally saline added to 25 ml. to give a 0·4% suspension of alexinated sheep cells. Such a suspension is clumped specifically by conglutinin or immuno-conglutinin.

Human sera examined

A total of 3282 human sera have been examined for immuno-conglutinin. Of this number 2800 were from apparently healthy persons and 482 were from persons suffering from clearly defined clinical diseases.

The sera from apparently healthy persons were obtained through the courtesy of the Cambridge Regional Blood Transfusion Service and these may be divided into two categories. First, a batch of 203 sera obtained in March and May 1954 from donors in the Cambridge area, and secondly, samples from Ipswich donors obtained at the monthly bleeding sessions from July 1954 to June 1956.

The sera from patients were all derived from in-patients in the United Cambridge Hospitals. This group was composed partly of an initial random sample taken from patients who were being venepunctured for other laboratory tests and partly of patients selected because the random survey suggested that interesting results might be expected in their clinical state. The results to be reported were derived from patients in whom the clinical diagnosis was certain.

An analysis of the source of the sera is given in Table 1.

RESULTS

Apparently healthy persons

It is not easy to ascertain or indeed specify what is the normal level of immuno-conglutinin in human serum. If immuno-conglutinin is, as we imagine, one of the physiological responses to an antigenic stimulus, there is really no such thing as

Table 1. *Human sera examined for immuno-conglutinin activity*

Classification	Number
A. Apparently healthy persons	
(i) Initial survey, Cambridge blood donors, March and May 1954	203
(ii) Ipswich blood donors—monthly sessions. July 1954–June 1956	2597
B. Hospital in-patients	
(i) Acute bacterial infections	95
(ii) Acute virus infections	17
(iii) Chronic bacterial infections	75
(iv) 'Rheumatism'	188
(v) 'Toxic states'	60
(vi) Control group of psychiatric hospital in-patients showing no physical abnormalities	47

a normal level, unless by normal level is inferred the basic level found in the serum of persons unexposed to an antigenic stimulus. Healthy persons are probably frequently being exposed to antigenic stimuli and also many apparently healthy persons may be in the process of combating a subclinical infection. On the other hand, the serum level of immuno-conglutinin found in the majority of apparently healthy persons should reflect the resting or basic level in human serum. The sera from many apparently healthy persons have now been examined to determine this norm of immuno-conglutinin titre and the scatter or distribution about this norm.

The first survey consisted of 203 apparently healthy persons who were bled during March and May 1954 in Cambridge by the Cambridge Regional Blood Transfusion Service. The results of the examinations on these sera are shown in Fig. 1*a*. In 52% of the sera there was either no conglutinin or a titre of 1 only. In 15%, however, the titre was greater than 4. Further analysis of the results according to the sex of donors showed no obvious difference.

To extend this preliminary survey 2597 sera have been examined from persons bled by the Cambridge Regional Blood Transfusion Service at their monthly bleeding sessions at Ipswich over the last two years. This has not only given us an extended overall picture but has also allowed us to observe variations in the distribution curve of the serum titres for the individual months. Of the total sera examined (Fig. 1*c*) 64% had either no immuno-conglutinin or a titre of 1 only, while the percentage having a titre in excess of 4 was about 13.

More information emerges if the results are viewed month by month (Fig. 2). There is considerable variation in the monthly distribution curves, but the majority show a norm with an absence of serum immuno-conglutinin. This is not true for the months July 1954 and April 1956, where the norm is shifted to the right. Some months, notably July, August and September 1954, show two norms. The distribution curves for the months April to August 1955 and December 1955 to February 1956 appear very constant. These monthly curves, together with the pooled results for the months of March to September 1955 (Fig. 1*b*), could perhaps be taken as

representative of the immuno-conglutinin distribution in a healthy population relatively free of antigenic stimulation with a norm of persons possessing no immuno-conglutinin. Here 76% of persons had either no immuno-conglutinin or a titre of 1 only, and only 5% had a titre above 4.

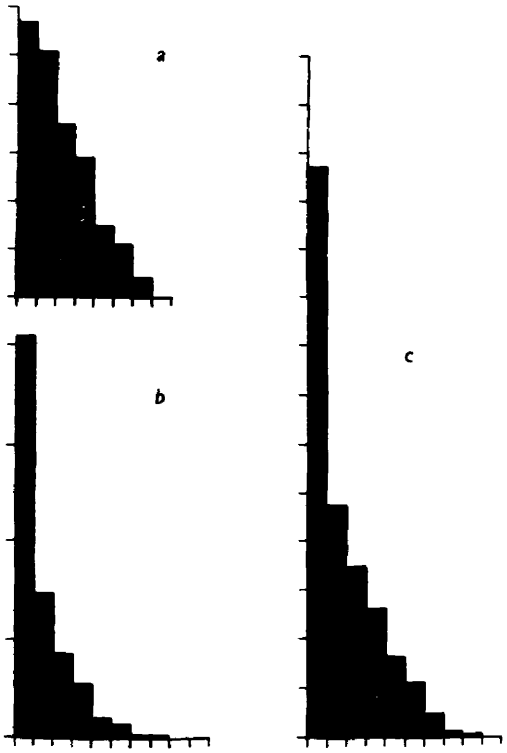

Fig. 1

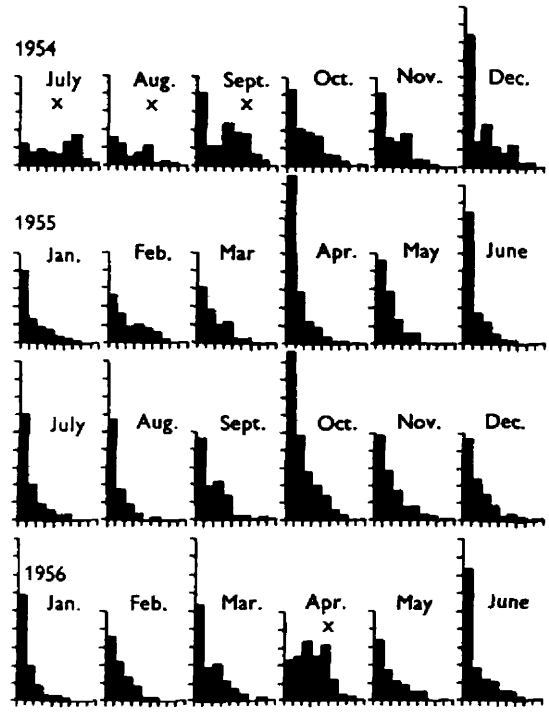

Fig. 2

Fig. 1. *a*. Immuno-conglutinin in the sera of 203 apparently healthy blood donors—March and May 1954, Cambridge. (One vertical division represents ten persons.) *b*. Immuno-conglutinin in the sera of 733 apparently healthy blood donors—March to September 1955, Ipswich. (One vertical division represents 100 persons.) *c*. Immuno-conglutinin in the sera of 2597 apparently healthy blood donors—July 1954 to June 1956, Ipswich. (One vertical division represents 100 persons.) Key to figures: horizontal divisions represent immuno-conglutinin titres 0, 1, 2, 4, 8, etc., up to 128; vertical divisions represent number of persons or sera—the scale varies in the individual figures.

Fig. 2. Immuno-conglutinin in the sera of apparently healthy blood donors taken at the monthly bleeding sessions at Ipswich from July 1954 to June 1956. x indicates months showing a marked deviation from what appears to be the normal mode of distribution. (One vertical division represents ten sera.)

As just mentioned, certain months (see Fig. 2) presented a marked deviation from the distribution curve shown in Fig. 1*b*. Especially noticeable in this respect were the months of July, August and September 1954 and April 1956. The months of July, August and September 1954 came at the commencement of these studies, and no attempt was made to correlate the high immuno-conglutinin levels with the state of health of these blood donors. But it is perhaps worthy of note that the summer of 1954 was very wet and cold and sore throats and colds were very

prevalent in the area concerned. An attempt is being made to find an explanation for the shift in the norm amongst blood donors for the month of April 1956 but as yet no conclusions are available. It is not, however, extravagant to suggest that this shift of the norm to the right reflects either a high incidence of minor clinical infections or a widespread asymptomatic virus infection in the majority of the local population.

To draw any conclusion from this section it is necessary to be somewhat conjectural. In the majority of healthy persons free from antigenic stimulation little or no immuno-conglutinin is detectable in the serum. On a population basis Fig. 1*b* is probably representative as a distribution curve for immuno-conglutinin in a healthy population. Any marked deviation from this curve probably reflects either a high incidence of minor clinical infections or an asymptomatic infection epidemic in the area.

Persons with various diseases

(1) *Acute bacterial infections*

Ninety-five serum samples from seventy-three patients with acute bacterial infections have been tested. The series included thirty cases of lobar pneumonia, sixteen cases of broncho-pneumonia, twelve cases of acute streptococcal tonsillitis, five cases of acute appendicitis, two cases of streptococcal septicaemia and two cases of acute meningitis. Many of the samples were taken at the time of the original diagnosis before antibiotics had been administered, but being relatively early in the disease one would not have expected a very marked antibody response by that time. Further serum samples obtained from these patients, and in some instances the initial samples, were taken after anti-bacterial drugs had been given. In this and subsequent sections not more than two samples were taken from any one patient.

The immuno-conglutinin levels found (Fig. 3*a*) cannot be taken as representative of the body's full immuno-conglutinin response to bacterial infection, for with modern diagnosis and therapy an acute untreated bacterial infection of more than a few days duration is a rarity. However, with the material studied 74 % of sera had a titre of 8 or greater (with healthy persons only 5 % had a titre of 8 or more, Fig. 1*b*) and of these five patients had a titre greater than 64.

The date of onset of symptoms could be fixed with reasonable accuracy in forty-five patients (sixty-one specimens), and in these patients the titre has been plotted against the day of illness on which the specimen was taken (Fig. 4). Up to the 9th day no titres greater than 16 were found. After the 9th day the majority of specimens showed a titre of 16 or greater.

(2) *Acute virus infections*

Seventeen sera have been tested from sixteen patients suffering from proven virus diseases. The majority of sera were from cases of poliomyelitis in the acute stage. The results (Fig. 3*b*) give the percentage of sera with a titre of 8 or more as 76. The titres have not been plotted against the probable day of the disease because of the small number involved.

(3) *Chronic bacterial infections*

The results of seventy-five examinations on sixty-one patients suffering from chronic bacterial infections are shown in Fig. 3*c*. Included in this group were twelve cases of tuberculosis predominantly of the chest, fifteen cases of bronchiectasis, thirteen cases with large chronic visceral abscesses, six cases with subacute bacterial endocarditis, four cases of active chronic osteomyelitis and two cases with lung abscesses. 98% of the sera had a titre of 8 or more and 33% had a titre of 64 or more.

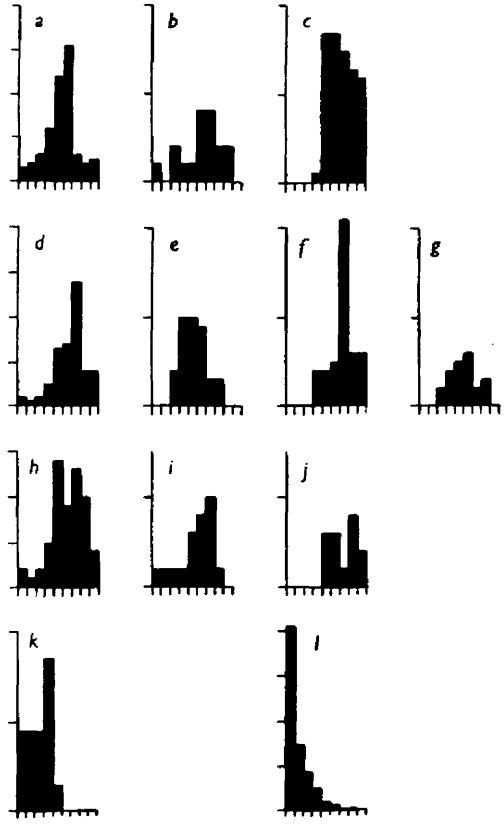

Fig. 3. *a*. Immuno-conglutinin in the sera of patients with acute bacterial infections. (One vertical division represents ten sera.) *b*. Immuno-conglutinin in the sera of patients with acute virus infections. (One vertical division represents five sera.) *c*. Immuno-conglutinin in the sera of patients with chronic bacterial infections. (One vertical division represents 10 sera.) *d–g*. Immuno-conglutinin in the sera of patients suffering from rheumatic disorders. (One vertical division represents ten sera.) *d*, acute rheumatoid arthritis; *e*, 'Burnt-out' rheumatism; *f*, acute rheumatic fever; *g*, mitral stenosis. *h*, *i*, *j*. Immuno-conglutinin in the sera of patients with so-called toxic diseases. (One vertical division represents five sera.) *h*, total cases examined; *i*, seventeeen cases of acute nephritis (included in *h*); *j*, thirteen cases of spondylitis (also included in *h*). *k*. Immuno-conglutinin in the sera of hospitalized psychiatric patients. These were examined to serve as some sort of control for the other hospitalized patients. (One vertical division represents ten sera.) *l*. Immuno-conglutinin in the sera of 733 apparently healthy blood donors—March–September 1955, Ipswich. Taken from Fig. 1*b* and brought to scale. (One vertical division represents 100 sera.)

(4) *Rheumatic disorders*

The material in this small survey consisted of 188 serum samples from 168 patients suffering from various forms of rheumatism. These were subdivided into the four following groups according to the clinical picture they presented: (1) *acute active rheumatoid arthritis*; (2) '*burnt-out*' *rheumatism* in which the patients showed no evidence of active disease but merely the end-results of previous active disease; (3) *acute rheumatic fever*; and (4) *mitral stenosis* in cases showing no evidence of acute rheumatic fever.

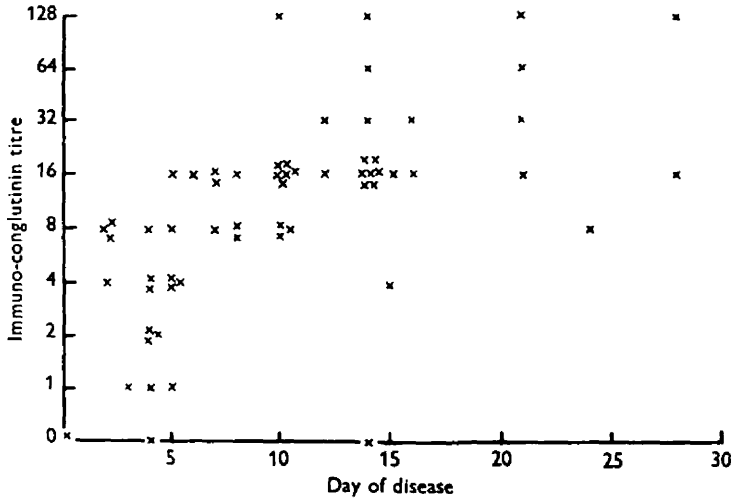

Fig. 4. Immuno-conglutinin in the serum of persons with acute bacterial infections plotted against the days after the first symptoms of the disease.

The immuno-conglutinin titres of the four groups are shown in Figs. 3*d*, *e*, *f* and *g*. In the *acute active rheumatoid arthritis* group the peak in the histogram occurred at a titre of 32 and 88 % of the samples had an immuno-conglutinin titre of 8 or above. With the '*burnt-out*' *rheumatism* group the peak occurred at a titre of 4–8 with 64 % of sera having a titre of 8 or higher. A similar relationship was found to exist between the other two groups.

(5) *Diseases associated with an antigen-antibody reaction*

Apart from the rheumatic groups of diseases there is a heterogeneous collection in which the tissue reaction associated with the disease is thought to be dependent on a primary antigen-antibody interaction. This group included acute nephritis, ankylosing spondylitis, anaphylactoid purpura and disseminated lupus erythematosus. The overall results on sixty sera from fifty-three patients are shown in Fig. 3*h*, together with separate histograms for the cases of acute nephritis and ankylosing spondylitis (Fig. 3*i* and *j*). Once again the immuno-conglutinin levels may be seen to be well above those found in healthy persons, 83 % having a titre of 8 or greater.

(6) *Psychiatric in-patients*

This group of psychiatric in-patients was included as a better control for the other hospitalized patients studied. The group consisted of forty-seven psychiatric patients in whom no physical abnormalitites could be found. Although this histogram does not resemble that shown in Fig. 1*b* characterizing healthy persons, yet only 6% of the sera had a titre of 8 or more.

DISCUSSION

The first conclusion to be drawn from this study is that, under certain conditions, immuno-conglutinin may be found in human sera to a high titre.

On examining individual sera from an apparently healthy population it was found that the majority of persons were without demonstrable immuno-conglutinin in their serum, while, on the other hand, a certain percentage of persons did have serum immuno-conglutinin mainly of low titre but in one or two cases to a quite substantial level (see Fig. 1*b*). During bacterial or virus infections immuno-conglutinin was regularly found in the serum and here most patients had a substantial titre (Fig. 3*a*, *b* and *c*). This situation is similar to that found experimentally in the rabbit.

From these findings it would seem reasonable to conclude that there is a resting state, as observed in the majority of healthy persons in which conglutinin is absent from the serum. It would not be correct to call this the normal level, as, according to the hypothesis we hold concerning immuno-conglutinin, its development is a normal physiological response to the adsorption and functioning of complement *in vivo*; also presumably a normal phenomenon.

In clinically healthy persons immune processes involving complement are probably frequently active warding off what turn out to be subclinical or asymptomatic infections. This probably accounts for the proportion of apparently healthy persons with low titres of immuno-conglutinin in their serum.

The variations, month by month, in the distribution curve of immuno-conglutinin in blood donors from Ipswich over a 2-year period are shown in Fig. 2. At the moment we can only speculate as to the reason for the deviations from the curve shown in Fig. 1*b* which we consider best representative for a group of healthy persons. The histograms for July, August and September 1954 (Fig. 2) show two modes or populations—one group of persons with immuno-conglutinin in the resting state and another group with a raised level of immuno-conglutinin and which we may conjecture was stimulated by common ailments such as tonsillitis and common colds. The histogram for April 1956 shows that the norm for this month was shifted to the right which rather suggests an asymptomatic infection epidemic in the local population. An attempt is being made to discover the stimulus responsible for this shift to the right.

In the diagnosed cases of bacterial or virus infections the majority of patients had an immuno-conglutinin titre of 16 or over. Had the diseases been allowed to run their natural courses the immuno-conglutinin response would probably have been more marked. However, with the available material, plotting the immuno-

conglutinin titre in each case against the day of the disease gave a graph (Fig. 4) which in its similarity to an antibody curve would support the contention that immuno-conglutinin is an antibody—an antibody reactive with the person's own complement.

The fact that the level of immuno-conglutinin was found to be raised in the rheumatic group of diseases and in those so-called toxic diseases where antigen-antibody interaction is incriminated in the aetiology is not only in keeping with, but is a consideration definitely supporting, the supposed underlying cause of these diseases. In the rheumatic group the immuno-conglutinin titre was higher in the acute phases than in the later stages.

It is not possible at present to say much on the significance of immuno-conglutinin either from the point of view of the patient or from the point of view of the investigator. It has been shown that immuno-conglutinin appears in the serum following either artificial antigenic stimulation or natural infection and a hypothesis has been put forward to explain its formation. It is suggested that during artificial immunization or infection antibody reacts with antigen and absorbs complement *in vivo*. It is also maintained that adsorbed complement has a configuration quite distinct from that of the same complement in solution, and that this new configuration of adsorbed complement is able to act auto-antigenically, producing immuno-conglutinin which is an antibody specific for adsorbed complement. What role this immuno-conglutinin plays in the immunity of the host has not as yet been studied, but its presence in human serum and the importance of understanding all the processes involved in immunity makes such a study of more than academic interest.

Until the exact role of immuno-conglutinin in immunity is known it is obviously not possible to assess its full value to the investigator or as a laboratory test. However, if in any particular disease of unknown origin a constantly raised level of immuno-conglutinin was found it would indicate that an antigen-antibody interaction was involved in the aetiology. It might also be found that a survey of immuno-conglutinin in a local population is a useful tool in epidemiological investigations. Any marked deviation from the normal distribution curve for immuno-conglutinin would indicate that a corresponding proportion of persons had recently experienced an antigenic challenge, presumably an infection, and thus an epidemic, symptomatic or asymptomatic, could be assumed without knowing the nature of the provocative agent.

SUMMARY

1. A survey has been made of the serum levels of immuno-conglutinin in apparently healthy persons.

2. The level of immuno-conglutinin is raised in infective diseases and in other diseases where an antigen-antibody interaction is incriminated in the aetiology.

3. The significance of immuno-conglutinin is discussed.

Without the technical assistance of Mrs C. Madin this work could not have been undertaken and we would like to record our appreciation. We would also like to

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