Circulating immune complexes in normal blood donors of three races

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Summary

Circulating immune complexes were measured by means of a modified Raji cell assay in 50 blood donors from each of three race groups — Indian, Black and White. The results ranged widely and were, on average, higher in Blacks than in others. The difference is probably related to the hypergammaglobulinaemia commonly found in Blacks. For maximum clinical usefulness, results should be expressed so as to indicate the degree of variation from the appropriate normal mean.


Having recently introduced the Raji cell assay for circulating immune complexes into our laboratory and being mindful of various immunological differences between the three population groups with which we deal, we felt it necessary to carry out the assay on a substantial number of normal sera before attempting to assess results in patients.

The Raji cell assay that we use is a modification of that described by Theofilopoulos et al. The test depends upon the fact that the membrane of these cells has strong receptors for bound C3 and IgG Fc, and hence will allow attachment of complexes, particularly those which have incorporated complement. Also important is the fact that Raji cells have no surface immunoglobulin, so that the test serum is the only possible source of the IgG detected.

Material and methods

Blood samples were collected from 50 normal blood donors in each population group — Black, White and Indian. The average age of the donors in the three groups was 31, 32 and 28 years respectively and the overall ratio of males to females was approximately 2:1. Most of the donors were either students or factory workers. All samples were collected between 09h00 and 12h00 and the serum was separated within 2 hours. Serum was immediately stored in small aliquots at -20°C, so that testing could be carried out on samples that had been thawed only once.

Raji cells propagated in RPMI 1640 + 10% fetal calf serum were washed three times, counted and resuspended in medium at a cell concentration of 2 x 10⁶/10 ml. Fifty microlitres of this suspension (2 x 10⁶ Raji cells) were used in each test. To this was added 25 µl of a 1-in-4 dilution of test serum in saline and the mixture was incubated at 37°C for 30 minutes. The cell pellet was then washed three times in saline containing 0,05% Tween 20. This was followed by enzyme-linked immunosorbent assay (ELISA) to detect IgG adhering to the surface of the cells. One millilitre of a 1-in-200 dilution (in saline-Tween 20) of rabbit anti-human IgG to which alkaline phosphatase had been previously conjugated was added. Incubation at 37°C for a further 2 hours was followed by three washes in saline-Tween 20. One millilitre of nitrophenyl phosphate solution (1 mg/ml) was then added to the disaggregated pellet of Raji cells. Colour change was allowed to proceed until a fairly deep yellow was noted in the high-reading control tube (usually after about 20 minutes). The reaction was then stopped by adding 100 µl of 10% sodium hydroxide. This addition was made to the tubes in the same order as that of the substrate, thus ensuring as far as possible an identical reaction time for enzyme and substrate in each tube. Finally, the tubes were centrifuged and the supernatant was decanted for reading in a spectrophotometer at 400 nm.

For quantitation of the results, a standard curve is made each day using serial dilutions of an aliquot of serum from a patient with systemic lupus erythematosus. This serum has been standardized against a known concentration of aggregated IgG and gives a reading (undiluted) equivalent to 9600 µg/ml of aggregated IgG. High-reading and low-reading control sera were also included with each test batch.

Levels of IgG, IgA, IgM and C3 in the samples were measured by laser nephelometry.

Results

Occasional very high readings (over 500 µg/ml) were obtained in each group; 1 Indian, 3 Blacks and 2 Whites fell into this category. We decided not to include these 6 among the 'normals'.

The results are graphically demonstrated in Fig. 1. The means were 75,4 µg/ml for Indians, 101,5 µg/ml for Blacks and 69,3 µg/ml for Whites, with respective standard deviations of 104,81 and 100 µg/ml.

![Fig. 1. Circulating immune complex levels in 150 blood donors, expressed in µg/ml, equivalent to aggregated IgG.](image-url)
In 75% of the Indians and 75% of the Whites the readings were below 100µg/ml, while only 50% of the readings from Blacks were below this figure.

The results for immunoglobulin and C3 are shown in Table I.

### TABLE I. MEAN IMMUNOGLOBULIN (Ig) AND C3 LEVELS (mg/d) OF 150 BLOOD DONORS

<table>
<thead>
<tr>
<th></th>
<th>IgG</th>
<th>IgA</th>
<th>IgM</th>
<th>Ig</th>
<th>C3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indians</td>
<td>1247</td>
<td>308</td>
<td>114</td>
<td>669</td>
<td>132</td>
</tr>
<tr>
<td>Blacks</td>
<td>1556</td>
<td>298</td>
<td>103</td>
<td>957</td>
<td>111</td>
</tr>
<tr>
<td>Whites</td>
<td>828</td>
<td>241</td>
<td>86</td>
<td>155</td>
<td>114</td>
</tr>
</tbody>
</table>

**Discussion**

The formation of immune complexes plays an important physiological role in self-protection and their presence in the circulation is indicative of an active humoral immune response designed to neutralize undesirable antigens or to lead to their destruction by phagocytes. Variable amounts of complexes will thus be found in the serum of normal people as the result of subclinical infections and infestations, or the absorption of antigens across mucosal barriers, or perhaps as the result of tardy phagocytosis.

Subjects who are deficient in secretory IgA, and whose surface defence mechanisms are therefore inadequate, regularly absorb unduly large amounts of bacterial and food antigens from the gut and consequently show increased levels of circulating immune complexes. It is also important to bear in mind that spontaneous aggregation of immunoglobulin molecules does take place in the circulation, and that such aggregates may exactly resemble antigen-antibody complexes in terms of particle size and biological activities, such as complement activation or adherence to suitable cell surface receptors. Since every one of the current assays for immune complexes depends upon one or other of these physical or biological properties, none of them is able to distinguish immunoglobulin aggregates from true complexes.

One further problem with the Raji cell assay in particular is that since the Raji cell is a B lymphocyte, antilymphocyte antibodies present in the test serum may react with it and produce falsely high readings in the test.

Because the finding of immune complexes in serum does not necessarily indicate disease, it is clearly vital that results be carefully interpreted in the light of the other laboratory and clinical features in each case.

Comparison between laboratories of results expressed in microgram equivalents of aggregated human gammaglobulin has little meaning because of inevitable variations in the quantitation method. Each laboratory should be in a position to compare results obtained in disease states with those from an appropriate control population, and undue emphasis on the microgram equivalents of gammaglobulin should be avoided.

Our results indicate that in apparently normal people a rather wide range (from below 10 µg/ml to about 400 µg/ml) of circulating complexes is found and that occasional very high figures, in the region of 1 mg/ml, will be encountered.

It is likely that spontaneous aggregation of immunoglobulins takes place more readily in hypergammaglobulinaimic states and we considered this the possible explanation for the 6 very high readings. For the 3 Black subjects in this category (total gammaglobulin levels of 2893, 3537 and 2024 mg/dl) this explanation is possibly correct. However, it does not explain the high readings from the 3 other subjects (1 Indian, 2 Whites) with gammaglobulin levels of 1059, 1018 and 1159 mg/dl.

No subject in this study was deficient in IgA.

The lack of an absolute standard of normal values calls for care in the reporting of results. In general, our practice now is to regard a figure below 100 as 'normal', one between 100 µg/ml and 500 µg/ml as 'mildly elevated', one between 500 and 1000 µg/ml as 'moderately elevated', and those above 1 mg/ml as 'markedly elevated'.

Most of the results in this study represent the mean of at least two observations and the reproducibility of our findings has been excellent in terms of the above classification. Finer analysis would be called for, we feel, when serial tests are done on the same subject, for example when assessing the effects of plasmapheresis in a patient with immune complex-mediated disease. Whenever possible, one of us makes a clinical assessment of the patient in order to assist with interpretation of the results.

In normal subjects with circulating immune complexes, it is probably the lack of complement activation which explains the absence of evidence of immune complex-mediated lesions. The C3 levels were normal in all subjects in the present series. Nevertheless, the interference of immune complexes with diverse cell functions, although less evident than their inflammatory effects, may also play an important role in some human diseases. Comparison of the results in the three race groups studied here shows that, on average, higher levels were encountered in Blacks. The relationship between these results and the hypergammaglobulinaemia prevalent in this group suggests either that ongoing antigenic stimuli are maintaining such levels of immune complexes or that spontaneous aggregation of IgG is occurring. At present we lack a test to make the distinction.

Results of immune complex estimations are often expressed as 'positive' or 'negative', but we feel that a classification such as ours, based upon comparison with appropriate controls and indicating the degree of deviation, is of greater clinical value.

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**REFERENCES**