Studies of lymphocytes in rheumatoid arthritis

I. Uptake of 125I-heat aggregated human IgG by Fc-receptor bearing lymphocytes

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SUMMARY The uptake of 125I-heat aggregated human IgG (125I-HAGG) by monocyte-free peripheral blood lymphocytes was investigated in patients with active rheumatoid arthritis (RA), inactive RA patients with other inflammatory joint diseases (ankylosing spondylitis and Still's disease), and in normal controls. The lymphocytes of patients with RA, whether active or inactive, showed significantly raised levels of 125I-HAGG uptake when compared with normals. Patients with other inflammatory joint diseases showed normal levels of HAGG uptake.

High uptake was not due to the presence of cytophilic antibodies and was not related to drug treatment, rheumatoid factor status, patient age, duration of disease, and did not correlate with disease activity. These results indicate that Fc-receptor bearing lymphocytes in patients with RA differ from those of normal subjects and patients with other inflammatory joint diseases. This difference was not due to differences in numbers of Fc-receptor bearing lymphocytes but may be due either to increased numbers of Fc-receptors on each cell or to increased avidity of such receptors for HAGG.

Abnormalities of the immune system are a common manifestation in patients with rheumatoid arthritis (RA). These abnormalities include production of antiglobulins (Natvig and Munthe, 1975), inhibition of leucocyte migration, and production of macrophage migration inhibitory factor by heat-aggregated gammaglobulin (HAGG) (Eibl and Sitko, 1975), and the presence of circulating immune complexes containing antiglobulins (Kunkel and Tan, 1964). These findings indicate abnormal responses to IgG but the immunological stimulus and cellular basis for these manifestations are unknown. Since no consistent differences in the number of Fc-receptor bearing lymphocytes have been found in the peripheral blood of patients with RA, these abnormalities could be due to differences in receptors for the Fc fragment of IgG on lymphocytes. These Fc receptors could either have increased avidity, or be present in increased numbers, or have an altered distribution on the cell surface. In order to test this possibility, the uptake of 125I-HAGG was measured in patients with RA, with other inflammatory joint diseases, and in normal controls.

Patients

Eighty-three subjects were classified into one of four groups. (1) Active rheumatoid arthritis: 27 patients were chosen who had an erythrocyte sedimentation rate (ESR) of 30 or more, a Ritchie index of 8 or more, and morning stiffness lasting over 1½ hours. (2) Inactive rheumatoid arthritis: 22 patients were chosen who had a normal ESR, a Ritchie index under 3, and no morning stiffness. (3) Other inflammatory joint diseases: 11 patients with ankylosing spondylitis and 3 patients with juvenile rheumatoid arthritis. (4) Controls: 8 subjects with low back pain and 12 laboratory staff.

Methods

LYMPHOCYTES

Lymphocytes were separated from 20 ml heparinised peripheral blood and were freed of phagocytic cells by incubation at 37°C with 150 µg/ml carbonyl iron before layering over a Triosil-Ficoll gradient (Bøyum, 1968). The cells were washed three times with 5% bovine serum albumin in phosphate-buffered saline (BSA/PBS) and adjusted to a concentration of 2 x 10⁶/ml. Monocyte levels were

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343
enumerated on cytosmears stained by the nonspecific acid esterase method (Lake, 1971). A total of 1000 cells were counted for the presence of phagocytic cells.

125I-LABELLING OF HAGG
Lyophilised human IgG (Cohn Fraction II) was radiolabelled with 125Iodine (Amersham, 125I as NaI, 100 mCi/ml) according to the method of McFarlane (1958). The 125IgG solution (200 µg/ml) was aggregated by heating to 63°C for 30 minutes. Oversized aggregate was removed by centrifugation at 12 000 g for 10 minutes. 125I-HAGG was stored at a concentration of 20 µg/ml in 90% fetal calf serum (FCS) at -20°C.

LYMPHOCYTE UPTAKE OF 125I-HAGG
Uptake of 125I-HAGG was investigated by resuspending purified lymphocytes (1 x 10^8) in 500 µl of 125I-HAGG solution (20 µg/ml in FCS). The cell suspension was incubated for 10 minutes at room temperature on a rotary mixer in plastic tubes (LP3, Luckams) which had been coated with 5% BSA/PBS at 37°C for 4 hours in order to block nonspecific uptake of 125I-HAGG. At the end of the incubation period, cells were washed three times with 5% BSA/PBS by centrifugation at 200 g for 4 minutes in order to remove unbound HAGG. Radioactivity due to 125I-HAGG bound to lymphocytes was measured in a LKB-Wallac gammacounter and expressed as counts per minute (cpm). True uptake was calculated by: (cpm of tubes incubated with lymphocytes and 125I-HAGG) - (cpm of tubes incubated with 125I-HAGG alone).

Experiments were performed to establish (a) the reproducibility of and (b) the variation in the method.

Subsequently test runs were performed with lymphocytes from patients and normals. Representatives from every group were included within each test in order to control any variation of uptake conditions; however the diagnostic category of individuals within the test was not known at the time of the experiment.

UPTAKE WITHIN RHEUMATOID PATIENT SUBGROUPS
A study was done to investigate any correlation between 125I-HAGG uptake and the drug treatment prescribed for the disease, the rheumatoid factor status of the patient, and the age of the subjects in the trial.

CYTOPHILIC ANTIBODY ASSAY
Lymphocytes which had been prepared from normal subjects were incubated for 30 minutes at 4°C in sera from patients with active RA who had a known high 125I-HAGG uptake. After washing with 5% BSA/PBS they were reassayed for 125I-HAGG uptake. As a control, lymphocytes from the same subject were simultaneously incubated in autologous normal and homologous normal sera.

Results

ENUMERATION OF PHAGOCYTIC CELLS
In all groups studied the proportion of phagocytic cells (monocytes and neutrophils) was similar, and <1/10^3 lymphocytes. It was therefore considered that pure preparations of lymphocytes were being used in these experiments.

REPRODUCIBILITY AND VARIATION IN THE METHOD
Repetitive trials; subject 1 (normal) had uptake results of 1.98 and 2.10 x 10^-2 µg/10^6 lymphocytes, measured at a 3-week interval. Subject 2 (RA active) had uptake results of 3.06 and 3.10 x 10^-2 µg/10^6 lymphocytes at a 2-week interval. The maximum observed variation in 125I counts per minute was ±4%.

125I-HAGG UPTAKE BY LYMPHOCYTES
Fig. 1 shows the results of 125I-HAGG uptake in four groups investigated. The uptake of aggregates by lymphocytes of normal subjects was 2.10 ± 0.31 x 10^-3 µg/10^6 cells. However, patients with inactive RA (uptake 3.08 ± 0.88 x 10^-2 µg) and active RA (uptake 3.55 ± 1.05 x 10^-2 µg) showed significant increases of uptake when compared to both the normal and other inflammatory disease groups.

![Graph showing uptake of 125I-HAGG by lymphocytes](http://ard.bmj.com/Ann Rheum Dis: first published as 10.1136/ard.37.4.343 on 1 August 1978. Downloaded from http://ard.bmj.com/)
(Table 1). The Wilcoxon rank sum test was used to compare the disease group populations to each other and to the normal group. Both RA groups showed highly significant increases in uptake from the normal and other inflammatory disease groups (P<0·01). There was no difference in uptake between patients with active or inactive RA (P = 0·10) No correlation was observed between 125I-HAGG uptake and drugs prescribed for RA patients (Fig. 2), patient age, or rheumatoid factor status.

**Presence of Cytophilic Antibody**

The results of the cytophilic antibody experiments are given in Table 2. The salient feature was that incubation of cells from normal subjects gave a significant serum from patients with active RA did not lead to increased HAGG uptake.

Table 1 Statistical analysis of results of 125I-HAGG uptake by lymphocytes in the various subject groups, using the Wilcoxon rank sum test

<table>
<thead>
<tr>
<th>Group</th>
<th>Controls</th>
<th>RA active</th>
<th>RA inactive</th>
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<tbody>
<tr>
<td>RA active</td>
<td>P&lt;0·01</td>
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<td></td>
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<tr>
<td>RA inactive</td>
<td>P&lt;0·01</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>IJD</td>
<td>NS</td>
<td>P&lt;0·01</td>
<td>P&lt;0·01</td>
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RA = rheumatoid arthritis; IJD = inflammatory joint disease; NS = statistically not significant.

Table 2 Cytophilic antibody investigation. The results of incubating peripheral blood lymphocytes from normal subjects for 1 hour at 40°C in autologous serum, homologous normal (N) serum, and homologous rheumatoid (RA) serum, on the subsequent uptake of 125I-HAGG. (Uptake expressed as μg × 10⁻³/10⁶ cells.) None of the uptake values is significantly different from the other.

<table>
<thead>
<tr>
<th>Preincubation medium</th>
<th>125I-HAGG uptake (mean ± SD)</th>
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<tr>
<td>Autologous N serum</td>
<td>1·72 ± 0·63</td>
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<tr>
<td>Homologous N serum</td>
<td>1·50 ± 0·43</td>
</tr>
<tr>
<td>Homologous RA serum</td>
<td>1·62 ± 0·57</td>
</tr>
</tbody>
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Discussion

The assay system for 125I-HAGG uptake used in this investigation provides a more quantitative assessment of aggregated IgG uptake by lymphocytes than previous experiments using fluorescent conjugated HAGG (Dickler and Kunkel, 1972). The method shows little deviation within a single subject preparation and proved to be reproducible.

HAGG may be taken up by macrophages and neutrophils as well as Fc-receptor bearing lymphocytes but phagocytic cells have been effectively removed from the system. Enumerations of such lymphocytes in the peripheral blood of patients with RA generally agree that there is no variation from normals (Froland et al., 1975; Brenner et al., 1975). We have also found (unpublished data by surface Ig immunofluorescent and EA-rosette formation that B cell populations are similar in the disease groups and normals. T-blast cells may carry Fc-receptors (Nussenzweig, 1975; Ryan et al., 1975), and raised numbers of blast cells have been found in the peripheral blood both of patients with active RA or active ankylosing spondylitis but not in patients with inactive disease (Bacon et al., 1975). However, since increased uptake was seen in RA patients with active or inactive disease and was not seen in ankylosing spondylitis, it is unlikely that these results are due to this mechanism. Recently, it has been suggested that the binding of immune complexes is performed by a third lymphocyte population, the L cells (Lobo and Horwitz, 1976; Froland et al., 1974), which lack membrane incorporated immunoglobulin but have receptors for membrane-labile cytophilic IgG. A variation of this third lymphocyte population might account for conflicting variations in T and B cell populations reported in rheumatoid arthritis. Similarly, the difference in 125I-HAGG uptake between patients with RA and the other groups of subjects seen in this study might be explained by a variation in the L cell population. However, in the
absence of published work on the proportion and numbers of L cells in the peripheral blood of rheumatoids, this explanation must be considered speculative.

It may be that rheumatoid factors or other antibodies present in RA serum adhere to lymphocytes so that a false Fc-receptor results (Winchester et al., 1974). This could cause an abnormally high HAGG uptake by RA lymphocytes. However, incubating normal lymphocytes with sera from RA patients whose cells had shown a high 125I-HAGG did not induce high 125I-HAGG uptake. Thus adsorption of cytophilic antibodies is also insufficient to account for our results.

We conclude therefore, that levels of HAGG uptake are due to differences in the Fc-receptor bearing lymphocytes in rheumatoid arthritis. Since there appears to be no difference in lymphocyte subpopulations in RA, this difference could be due to either increased numbers of Fc receptors per cell, or increased avidity of the receptors for aggregate. Experiments using Scatchard plots (1958) are in progress to determine which of these two possibilities is correct. Fc-receptor differences might be due to a change in surface characteristics, for example through viral infection, or may be genetically determined. The similar degree of aggregated uptake by rheumatoid lymphocytes whether the disease is active or inactive, indicates that this quantitative difference is probably not a consequence of disease activity. It is difficult to differentiate between a virally-induced change in Fc-receptor behaviours and a change due to genetic mechanisms, since the former would still be present even during remission of disease. However, genetic studies in families should answer this question. It is not clear whether Fc-receptors are identical with Ia antigens (Dickler and Sachs, 1974), or closely associated structures on the lymphocyte surface (Schirrmacher et al., 1975; Kerbel, 1975). Since such an association appears to be true for the human HLA system (Solheim et al., 1976) this is especially interesting because of our recent demonstration of the association of a specific B cell alloantigen in RA (Panayi and Woolley, 1977). Thus, in future studies it will be possible to follow in the same family aggregate uptake, presence of the disease, and the expression of B cell alloantigens.

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References


