Studies of lymphocytes in rheumatoid arthritis

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

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SUMMARY The uptake of ^{125}I -heat aggregated human IgG (^{125}I -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 ^{125}I -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 ^{125}I -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 $2 \times 10^6/\text{ml}$. Monocyte levels were

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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 (1×10^6) 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 $<1/10^8$ 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 2.10×10^{-2} µg/ 10^6 lymphocytes, measured at a 3-week interval. Subject 2 (RA active) had uptake results of 3.06 and 3.10×10^{-2} µg/ 10^6 lymphocytes at a 2-week interval. The maximum observed variation in ¹²⁵I counts per minute was $\pm 4\%$.

¹²⁵I-HAGG UPTAKE BY LYMPHOCYTES

Fig. 1 shows the results of ¹²⁵I-HAGG uptake in the four groups investigated. The uptake of aggregate by lymphocytes of normal subjects was $2.10 \pm 0.10 \times 10^{-2}$ µg/ 10^6 cells. However, patients with inactive RA (uptake $3.08 \pm 0.88 \times 10^{-2}$ µg) and active RA (uptake $3.55 \pm 1.05 \times 10^{-2}$ µg) showed significant increases of uptake when compared to both the normal and other inflammatory disease groups

Fig. 1 Uptake of ¹²⁵I-heat aggregated human IgG (¹²⁵I-HAGG) by phagocyte-free peripheral blood lymphocytes in patients with rheumatoid arthritis (RA), other inflammatory joint diseases (IJD), and normals. Each point is the mean of triplicate observations for each subject. The rectangle represents the uptake value (mean $\pm$ 2SD) for the normal group.

(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 ^{125}I -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 in homologous serum from patients with active RA did not lead to increased HAGG uptake.

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

	Controls	RA active	RA inactive
RA active	$P<0.01$		
RA inactive	$P<0.01$	NS	
IJD	NS	$P<0.01$	$P<0.01$

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 ^{125}I -HAGG. (Uptake expressed as $\mu\text{g} \times 10^{-2}/10^6$ cells.) None of the uptake values is significantly different from the other

Preincubation medium	^{125}I -HAGG uptake (mean $\pm$ SD)
Autologous N serum	1.72 ± 0.63
Homologous N serum	1.50 ± 0.43
Homologous RA serum	1.62 ± 0.57

Discussion

The assay system for ^{125}I -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, 1974; 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 ^{125}I -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

Fig. 2 Uptake of ^{125}I -HAGG by rheumatoid lymphocytes from patients taking different drugs. NSAID = nonsteroidal anti-inflammatory drugs (aspirin, indomethacin, phenylbutazone). The rectangle represents the uptake value (mean $\pm$ 1SD) for the patients receiving no drugs.

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 ^{125}I -HAGG did not induce high ^{125}I -HAGG uptake. Thus adsorption of cytophilic antibodies is also insufficient to account for our results.

We conclude therefore, that raised 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 (Schirmacher *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 Wooley, 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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