Lymphocytes bearing Fc γ receptors in rheumatoid arthritis. I. An increased subpopulation of cells in rheumatoid arthritis detected with Facb rosettes

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SUMMARY The chronic production of IgM and IgG antiglobulins is a major feature of rheumatoid arthritis. This implies an abnormal interaction between rheumatoid leucocytes and IgG. A novel rosette assay employing rabbit Facb-coated calf red blood cells has been developed to study receptors for IgG on peripheral blood lymphocytes. Cells were obtained from groups of patients with rheumatoid arthritis (RA), osteoarthritis (OA), ankylosing spondylitis (AS), and healthy control subjects. Receptors for Facb were found on an increased proportion of lymphocytes from RA patients compared with the other groups tested. It has been shown that the Facb rosette assay detects a subpopulation of lymphocytes bearing receptors for the Fc region of IgG. This receptor is clearly capable of recognising and binding only the C γ 2 domain within the Fc region. As such it shows different specificity from some other Fc receptors detected on mononuclear cells. The number of Facb rosette-forming lymphocytes in an individual sample correlated well with the number of cells bearing 'high avidity' Fc receptors. However, the incidence of these cells in RA patients could not be correlated with disease activity, disease duration, or levels of IgM and IgG rheumatoid factor. Thus increased Facb rosette cells may represent a fundamental imbalance of the immune response in patients with rheumatoid arthritis.

The commonest immunological abnormality associated with rheumatoid disease is the presence in a large majority of patients of antibodies directed against epitopes in the Fc region of IgG—rheumatoid factors. The cellular mechanisms underlying this phenomenon are still unknown, but much evidence has accumulated to support an abnormal interaction between rheumatoid leucocytes and IgG. Several authors have demonstrated specific migration inhibition of rheumatoid cells induced by both native and heat-aggregated IgG¹⁻³ and by IgG-IgG immune complexes.⁴ Rheumatoid mononuclear cells have been shown to produce migration-inhibitory-factor (MIF)-like material when cultured with immune complexes.⁵ Increased binding of IgG by rheumatoid lymphocytes has been demonstrated with either ¹²⁵I-labelled heat-aggregated IgG⁶ or antibody-coated erythrocytes (EA rosettes).⁷⁻⁹

Little is known of the specificity or cellular distribution of Fc receptors (FcR) involved in the above reaction. It has been shown that rheumatoid leucocytes are specifically inhibited in a migration assay by rabbit Facb, a fragment of IgG which does not contain the terminal C γ 3 domains.⁴ This finding indicates that some rheumatoid cells express receptors with binding specificity for the C γ 2 domain of IgG. In this report a novel rosette assay utilising bovine erythrocytes coated with the Facb fragment of rabbit IgG has been used to identify this population.

Patients and methods

Peripheral blood was obtained from 3 groups of patients and from a control group of healthy hospital personnel. Patients with rheumatoid arthritis were being treated with nonsteroidal anti-inflammatory drugs only. Blood samples were also obtained from patients with ankylosing spondylitis attending an inpatient physiotherapy course at the Royal National Hospital for Rheumatic Diseases and from patients with osteoarthritis of the hip or knee.

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LYMPHOCYTES

Mononuclear cells were obtained from heparinised peripheral blood by density flotation on Ficoll-Paque (Pharmacia).¹⁰ In some experiments phagocytic cells were removed by preincubation of the blood with 20 mg/ml carbonyl iron for 30 minutes at 37°C. The interface cells were harvested, washed 3 times with phosphate-buffered saline (PBS), and adjusted to a concentration of 2×10^6 per ml. Monocytes were enumerated by staining for non-specific esterase.¹¹

PREPARATION OF IgG AND facb

Rabbit anticalf red blood cell serum was kindly provided by Dr J. Smith, Tenovus Research Laboratories, Southampton. IgG was purified from the rabbit antiserum by affinity chromatography on protein A-Sepharose CL-4B (Pharmacia). Facb fragment was obtained from this IgG by plasmin digestion as described by Stewart *et al.*¹² Final purification of the Facb from any residual IgG was achieved by chromatography on protein A-Sepharose CL-4B.

EA AND facb ROSETTE ASSAYS

Calf blood in Alsever's solution was obtained either from the Tenovus Research Laboratories, Southampton, or from Tissue Culture Services Ltd. (Slough). The red blood cells were washed 3 times with PBS and adjusted to a 2% v/v suspension. They were sensitised by incubation with an equal volume of the appropriate serial dilution of IgG or Facb for 30 minutes at 37°C. After a further 3 washes in PBS the coated erythrocytes were adjusted to a 1% v/v suspension. Equal volumes (200 μl) of this preparation and the lymphocyte suspension were mixed in plastic tubes (LP3, Luckham) and immediately centrifuged. The tubes were incubated for 60 minutes at 4°C, and the pellets were then carefully resuspended on an inclined turntable (20 rpm, 1 minute). After the addition of a small volume of crystal violet solution to aid counting of the lymphocytes the suspension was placed in a haemocytometer and the rosettes enumerated. A rosette was defined as 3 or more red cells adhering to a lymphocyte and 200 cells were scored to obtain a percentage rosette figure. Lymphocytes from at least 1 healthy control were included in each batch of assays.

Results

Results of experiments using the EA rosette assay to detect FcR on rheumatoid and control mononuclear cells are given in Fig. 1. An increased percentage of rheumatoid cells expressing FcR compared with control cells was noted at all dilutions

tested. This difference was highly significant at all IgG titres between 1/400 and 1/3200. The 1/200 dilution was the maximum subagglutinating titre of IgG coating with this particular preparation. At this dilution 36% of RA cells and 28% of control cells formed EA rosettes.

Parallel experiments were carried out to detect Facb rosetting cells in the above mononuclear cell suspensions. The results are presented in Fig. 2.

Fig. 1 EA rosette formation by rheumatoid and healthy mononuclear cells. The columns and bars represent the mean $\pm$ 1 standard deviation %EA rosettes at various dilutions of IgG sensitising the calf erythrocytes. The numbers of samples tested were as follows: (IgG antibody titre⁻¹: RA/healthy) 200: 8/8; 400: 10/10; 800: 10/10; 1600: 34/20; 3200: 26/19; 6400: 7/7. EA rosette formation by RA and healthy cells was compared at each IgG dilution by Student's t test: 200: not significant (NS); 400: $P < 0.02$; 800: $P < 0.005$; 1600: $P < 0.001$, 3200: $P < 0.001$; 6400: NS.

Fig. 2 Facb rosette formation by rheumatoid and healthy mononuclear cells. The columns and bars represent the mean $\pm$ 1 standard deviation % Facb rosettes at various dilutions of Facb sensitising the calf erythrocytes. The numbers of samples tested were as follows: (Facb titre⁻¹: RA/healthy) 100: 6/6; 128: 27/18; 200: 9/8; 400: 3/2. Facb rosette formation by RA and healthy cells was compared at each Facb dilution by Student's t-test 100: NS; 128: $P < 0.001$; 200: NS; 400: NS.

Again the rheumatoid cells showed increased rosette formation compared with healthy controls over a range of Facb dilutions. This difference was highly significant at the 1/128 dilution ($P<0.001$) but failed to reach statistical significance at 1/100 because of the small numbers of patients tested ($n=6$). A comparison of Figs. 1 and 2 shows that Facb rosettes detected a smaller proportion of peripheral blood mononuclear cells than EA rosettes. In some experiments the mononuclear cells were depleted of monocytes by incubation of the blood with carbonyl iron. This procedure reduced monocytes to less than 0.5% of the total cells as judged by non-specific esterase staining. The incidence of Facb rosetting cells was not changed by the carbonyl iron treatment, although the total EA rosetting population was reduced (Table 1).

The percentages EA and Facb rosetting lymphocytes were compared in individual samples of rheumatoid and healthy cells. The results are shown in Fig. 3. It was found that there was a good correlation ($r=0.784$) between percentage EA rosettes at 1/1600 (from Fig. 1) and percentage Facb rosettes

at 1/128 (from Fig. 2). The incidence of Facb rosetting cells was also determined in peripheral blood from patients with osteoarthritis and ankylosing spondylitis and these results compared with RA and healthy subjects (Fig. 4). Neither the OA nor AS group showed a statistically significant difference in Facb rosetting cells from healthy controls. However, although the RA group was the only one to differ significantly from controls ($P<0.001$), occasional samples with increased

Table 1 Effect of monocyte depletion on EA and Facb rosette formation

Subject	% EA rosettes		% Facb rosettes	
	Pre	Post	Pre	Post
1	18	11	6	5
2	30	23	5	5
3	30	16	7	6
4	19	14	5	7
5	23	17	5	5

Peripheral blood mononuclear cells from 5 RA patients were assayed for EA and Facb rosette formation before and after depletion of mononuclear phagocytes by incubation with carbonyl iron.

Fig. 3 Correlation between % EA rosettes and % Facb rosettes in individual samples of RA and control mononuclear cells. Linear regression analysis was performed on the individual % EA rosette values at 1/1600 IgG dilution (Fig. 1) and the corresponding % Facb rosette values at 1/128 Facb dilution (Fig. 2). The data fit the straight line $y=0.789x + 2.345$ ($r=0.784$).

Fig. 4 Facb rosette formation by mononuclear cells from healthy controls, osteoarthritis, ankylosing spondylitis, and rheumatoid arthritis patients. The dilution of Facb used to sensitise the erythrocytes was 1/128 (Fig. 2). The solid horizontal bar represents the mean % Facb rosette formation in each group and the hatched bars represent 1 standard deviation from the mean. Groups of data were compared by Student's *t* test: Healthy versus OA (NS); healthy versus RA ($P<0.001$); healthy versus AS (NS).

Fab rosettes were seen in all groups. The AS patients with high percentage Fab rosettes tended to be those with peripheral joint involvement. Attempts were made to correlate the incidence of Fab rosetting cells in RA peripheral blood with clinical and laboratory assessments of disease activity. No correlation was found between percentage Fab rosettes and any of the other parameters studied, namely, clinical assessment, synovial inflammation measured by quantitative thermography, disease duration, plasma viscosity, IgM or IgG rheumatoid factor titre. High values of percentage Fab rosettes were often noted in patients with early RA, that is, within 1 year of diagnosis.

Discussion

All 3 major groups of lymphocytes have been shown to contain FcR-positive cells. Thus, FcR has been demonstrated on some T cells¹³ and on B cells.¹⁴ These receptors have also been shown on the non-T, non-B 'third cell' population,¹⁵ called UL cells by Dickler¹⁶ and L cells by Horwitz and Lobo.¹⁷ Recently a number of techniques for detecting FcR have been applied to human lymphocytes in an attempt to distinguish the various subpopulations expressing these receptors.¹⁸⁻²⁰ Such studies suggest that FcR on B cells are less readily detectable than FcR on other lymphocytes.^{21,22} However, these studies have all been carried out with healthy human lymphocytes, and such data may not be directly comparable to the properties of FcR on rheumatoid cells.

The Fab rosette assay described in this paper is a novel technique for the detection of a subpopulation of lymphocytes with receptors for IgG. This subpopulation is greater in the peripheral blood of patients with rheumatoid arthritis than in that of patients with osteoarthritis or ankylosing spondylitis or of healthy control subjects. The number of Fab rosetting cells correlated well in individual patients with the number of cells forming EA rosettes under suboptimal conditions, that is, with low concentrations of IgG coating the calf erythrocytes. Such lymphocytes are often referred to as expressing 'high avidity FcRs', though strict measurements of receptor avidity for IgG have not been performed on these cells. From our results Fab rosettes appear to be a marker for a subgroup of lymphocytes within the FcR-positive population. The receptor clearly has specificity for the Cy2 region of IgG.

In this regard at least the receptor recognising Fab may be similar to that detected by human EA (Ripley) rosette formation.²³ It is not clear from the

present study whether Fab rosette-forming cells constitute a discrete subpopulation of lymphocytes with definite chemical and biological properties. Further characterisation of these cells and the receptor is in progress.

Depletion of phagocytes by treatment of the blood with carbonyl iron suggests that monocytes do not form Fab rosettes. This is in agreement with other work showing that the binding of IgG to monocytes involves the Cy3 domains.^{24,25} The use of Fab to detect FcR also precludes any involvement of cytophilic antiglobulins in rosette formation, since rabbit Fab has been shown not to bind to rheumatoid factor.¹² In addition, incubation of the lymphocytes with trypsin before Fab rosette formation has been shown not to reduce the number of rosettes detected (unpublished data). Thus the assay is directly applicable to FcR determination on lymphocyte subpopulations and overcomes potential interference from contaminating monocytes and rheumatoid factor.

The significance of the abnormal FcR distribution on rheumatoid lymphocytes detected by Fab rosette formation has yet to be established. A number of other workers have demonstrated increased FcR-bearing mononuclear cells in RA using a variety of techniques.⁶⁻⁹ These authors were unable to show any association between increased FcR expression and various disease parameters. In agreement with the above studies the incidence of Fab rosetting cells did not correlate with clinical and laboratory assessments of disease activity. FcR-positive lymphocytes appear therefore not to be involved in mechanisms directly affecting disease activity. It is possible that these cells represent part of the overall response of the immune system to an event early in the disease process. Alternatively, FcR expression may be associated with the genetic factors underlying the development of RA in susceptible individuals.

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