# Peripheral T Lymphocytes from Rheumatoid Arthritis Patients Recognize Determinants on Light Chains and/or Fab Fragments of Immunoglobulin G

N. RADOIU, R. P. CLEVELAND, and M. A. LEON, *Department of Immunology and Microbiology, Wayne State University School of Medicine, Detroit, Michigan 48201; Arthritis Clinic, Metropolitan Hospital, Detroit, Michigan 48206*

ABSTRACT Peripheral blood T lymphocytes from 29 of 31 patients with rheumatoid arthritis incorporated significant quantities of thymidine when cultured with pooled human immunoglobulin G (IgG). In contrast to the observation of general reactivity to pooled IgG, responses to pooled IgM were rare (3 of 26 patients). None of 11 controls responded to either IgG or IgM. Response to IgG is maximal on day 6 of culture and is dependent on concentration of IgG. The responding cells recognize determinants on monoclonal light chains and/or Fab fragments. Response to light chains follows one of three patterns: preferential response to lambda chains, preferential response to kappa chains, and essentially equal response to either kappa or lambda chains.

## INTRODUCTION

Although T cells constitute a major component of the rheumatoid lesion (1–3), their function and immunological specificity have not been clearly established. Models, developed from experimental studies of immune responses to multideterminant protein antigens, ascribe essential roles to T cells in providing both helper and suppressor activities that regulate such responses (4–6). If rheumatoid factor (RF),[1] an autoanti-IgG, is representative of immune responses to protein antigens, by analogy, T cells should regulate RF production. Furthermore, such regulatory T cells should possess receptors recognizing determinants on IgG and respond to those determinants under appropriate in vitro culture conditions. These considerations prompted us to examine the in vitro proliferation responses to IgG of T lymphocytes derived from patients with rheumatoid arthritis (RA). The results presented in this report confirm the theoretical prediction that T cells from patients with circulating RF respond significantly to in vitro stimulation by IgG, and further, that the response is directed towards determinants present on Fab and/or light chains.

## METHODS

IgG and IgM immunoglobulin fractions were isolated from pooled normal sera by column chromatography on DEAE-cellulose (Whatman DE-52, Whatman Inc., Paper Div., Clifton, NJ) and gel filtration of Sephacryl-200 (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, NJ). IgG from a single rheumatoid serum was isolated in a similar manner. The purity of IgG and IgM at 3 mg/ml was determined by immunodiffusion with specific anti-IgG and anti-IgM sera. Fab and Fc fragments were prepared by papain digestion of the pooled normal IgG fraction (7) and separated on a Sephacryl-200 column. The fragments were identified by immunodiffusion using anti-Fab and anti-Fc sera. Light chains, Bence Jones proteins (1 kappa, 1 lambda) from two patients with multiple myeloma, as well as human $\beta$2-microglobulin, were generously provided by Dr. David Poulik. Purity was assessed on sodium dodecyl sulfate (SDS)-polyacrylamide gels by the method of Laemmli (8). Each light chain preparation migrated as a single band with a mol wt of $\sim$25,000 when compared with standard molecular weight markers. There was no evidence for significant contamination with $\beta$2-microglobulin. On the other hand, the $\beta$2-microglobulin preparation separated into two components, a major band migrating with a molecular weight typical of $\beta$2-microglobulin (12,000) and a minor component, comprising <5% of the total protein, which showed a mobility close to that corresponding to light chain.

Lymphocytes were isolated from peripheral blood by a modified Ficoll-Hypaque method (9) and the cells were separated on nylon wool columns (10, 11). The distribution of markers in these preparations is given in Table I. The nonadherent fraction was designated the T cell fraction whereas

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[1] *Abbreviations used in this paper*: PWM, pokeweed mitogen; RA, rheumatoid arthritis; RF, rheumatoid factor.

TABLE I

*Distribution of Markers on Nylon Wool-Passaged Peripheral Blood Leukocytes*

| Cell fraction* | OKT3+ | Ig+ | Esterase+‡ | Null | Recovery |
|---|---|---|---|---|---|
| | | | | | % |
| Unseparated | — | — | 13% | — | — |
| Nonadherent | 80–91% | 1–3% | 1% | 5–18% | 40% |
| Adherent | 60–61% | 16–28% | 1% | 10–23% | 8%§ |

* Mononuclear leukocytes from peripheral blood were prepared on Ficoll-Hypaque gradients and separated into nylon wool adherent and nonadherent fractions as described in Methods. The distribution of cellular markers in each fraction was assessed by direct immunofluorescence for OKT3 and surface immunoglobulin and by nonspecific esterase activity for esterase-positive cells. Null cells were assessed by difference from 100%.

‡ Single determination.

§ Because significant numbers of cells remained trapped in the nylon wool these percentages of markers do not give a true picture of the distribution in the total adherent fraction.

the recovered adherent fraction was designated the B cell fraction, although the latter is only enriched for B cells relative to the nonadherent fraction. Esterase-positive cells were measured as described (12). Cells showing positive reactivity with OKT3 antiserum were enumerated according to directions of the vendor (Ortho Pharmaceutical, Raritan, NJ). Cells positive for surface Ig were enumerated using fluorescein-labeled $(Fab')_2$ fragments of sheep antihuman Ig (Miles Laboratories, Inc., Ames Div., Elkhart, IN). Isolated lymphocytes ($2 \times 10^5$ cells in 200 $\mu$l of RPMI 1640 containing 5% heat-inactivated fetal bovine serum, penicillin 40 U/ml, and streptomycin 40 $\mu$g/ml) enriched for T or B cells were incubated in flat-bottomed microculture plates (Falcon Labware, Div. of Becton Dickinson & Co., Oxnard, CA) with 10 $\mu$l of either normal saline, 1:20 pokeweed mitogen (PWM) (Gibco Laboratories, Grand Island Biological Co., Grand Island, NY), 250 $\mu$g/ml normal or rheumatoid IgG, normal IgM, Fab, Fc, or light chains, at 37°C in 5% $CO_2$ and 95% air in a water-saturated incubator for 6 d. On the day of harvesting, cultures were pulsed with 0.5 $\mu$Ci of [$^3$H]thymidine (6.7 Ci/mM, New England Nuclear, Boston, MA) and reincubated for 4 h. Cultures were harvested on glass fiber filters (Whatman GF/C, Whatman Inc., Paper Div.) and counted in a scintillation counter. The results are expressed as stimulation index (counts per minute with reagent/counts per minute with saline). Each experimental point represents the mean counts of six cultures. Significance was calculated using Student's *t* test.

The patients were all seropositive with definite rheumatoid arthritis (criteria of the American Rheumatism Association) receiving no medication except for buffered aspirin (4–5 g/d). Controls were healthy seronegative laboratory personnel.

## RESULTS

*Response of peripheral blood lymphocytes from rheumatoid patients.* To determine whether lymphocytes specifically reactive with human IgG could be demonstrated in patients with RA, the in vitro proliferative responses of T cell-enriched or B cell-enriched fractions of peripheral blood lymphocytes were examined. Each fraction was cultured separately with IgG isolated from either a pool of normal human serum or from a single rheumatoid patient. Stimulation with PWM was included in all experiments as a positive control and all cell suspensions responded well to this mitogen (data not shown). As shown in Fig. 1, lym-

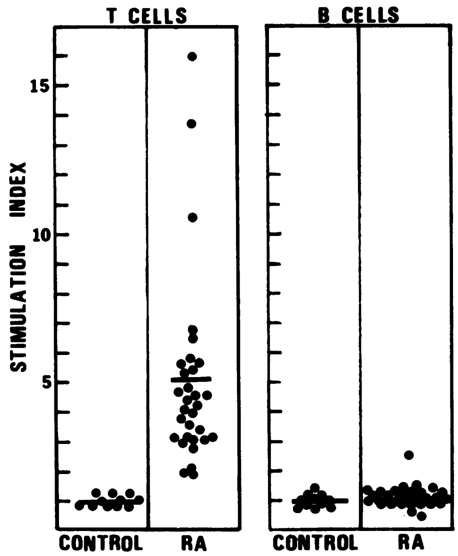


FIGURE 1 Stimulation of peripheral blood lymphocytes by human IgG. Peripheral blood lymphocytes from normal individuals or patients with RA were separated into T cell- or B cell-enriched fractions on nylon wool. Each fraction ($2.0 \times 10^5$ cells/0.2 ml) was stimulated in vitro with 10 $\mu$l of pooled normal human IgG (250 $\mu$g/ml). All cultures were harvested on day 6. Each point represents the stimulation index (counts per minute in cultures with antigen/counts per minute in cultures without antigen) of a single individual. The mean stimulation index for each group is indicated by the bar.

phocytes (both T cell and B cell fractions) from 11 normal individuals did not respond to normal IgG. In contrast, peripheral blood T cells from rheumatoid patients incorporated significant amounts of [$^3$H]thymidine ($P < 0.001$) in the presence of normal IgG. Similar results were obtained with rheumatoid IgG (data not shown). B cell fractions from rheumatoid patients did not respond to the same stimulants. In similar experiments, IgM, isolated from normal individuals, induced significant incorporation of [$^3$H]-thymidine in only 3 of 26 patients (Fig. 2).

*Dose dependence of response to IgG.* T cells isolated from a patient with rheumatoid arthritis were cultured in vitro with various concentrations of normal human IgG or human β2-microglobulin. The results, presented in Fig. 3, indicate that IgG at concentrations of 125–250 μg/ml stimulated maximal thymidine incorporation. In addition, no significant stimulation occurred in the presence of human β2-microglobulin, even at the low levels that could contaminate the light chain preparations used in subsequent experiments. Similar results were observed with T cells from seven other patients. T cells from normal individuals did not respond to stimulation with IgG over the range 50 to 1,000 μg/ml. Dose response data were not obtained for fragments of IgG or light chains (*vide infra*), which were arbitrarily used at 250 μg/ml.

*Kinetics of response to IgG.* Peripheral blood leukocytes isolated from a patient with RA were cultured in vitro with normal human IgG. Incorporation of thymidine was measured on days 4, 5, 6, and 7. The results

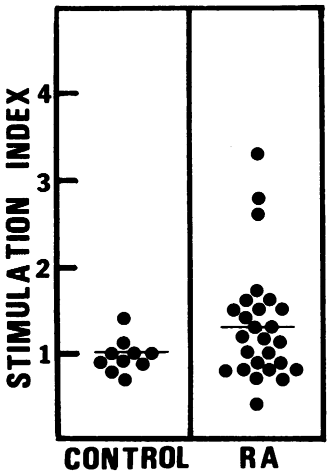


FIGURE 2 Stimulation of peripheral blood T cells by human IgM. Peripheral blood T cells from normal individuals or patients with RA were isolated as described in Fig. 1 and cultured with 10 μl pooled normal human IgM (250 μg/ml). Each point represents the stimulation index of a single individual. The mean stimulation index for each group is indicated by the bar.

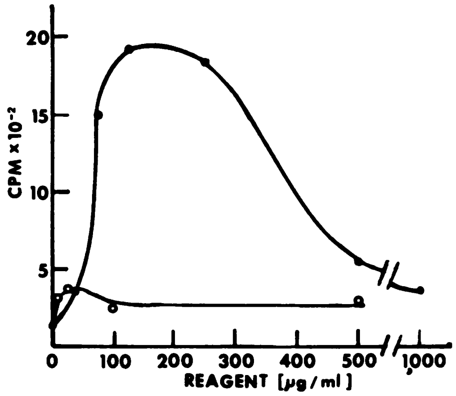


FIGURE 3 Dose dependence of proliferative response of peripheral blood leukocytes, from a patient with RA, to human IgG or human β2-microglobulin. Peripheral blood leukocytes were cultured with 10 μl of various concentrations of human IgG (●) or β2-microglobulin (O) for 6 d.

(Fig. 4) demonstrate that incorporation of thymidine increased slowly during the first 5 d of culture, reached a peak on day 6, and declined on day 7. Similar results were obtained with cells from two other patients.

*Response to subfractions of human IgG.* To define more clearly the specificity of the responses, T cells from normal individuals or from patients with RA were cultured in vitro with Fab, Fc, or monoclonal lambda or kappa light chains. In no instance did T cells from normal individuals respond. In contrast, T cells from all RA patients tested responded to determinants present on Fab and isolated kappa or lambda light chains. Fc fragments induced little or no re-

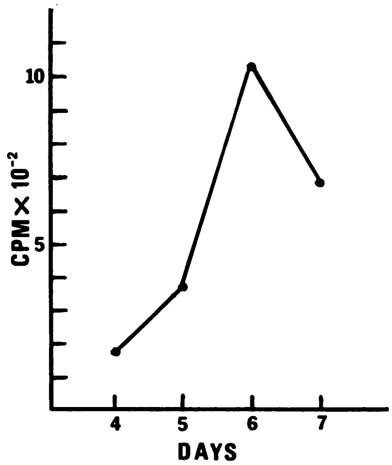


FIGURE 4 Kinetics of proliferative response of peripheral blood leukocytes from a rheumatoid patient to human IgG. Each point represents the difference between the mean counts per minute in cultures with antigen minus the mean counts per minute in cultures without antigen.

sponse. As shown in Table II, five patients demonstrated similar reactivity to both lambda and kappa light chains. Of the remaining five patients, four responded preferentially to stimulation with lambda chains and one responded preferentially to kappa light chains.

## DISCUSSION

The results presented in this report indicate that either normal or rheumatoid IgG stimulated significant in vitro responses by peripheral T lymphocytes from RA patients. Peripheral T lymphocytes from controls fail to respond under the same conditions. Of the 31 patients tested, 29 demonstrated stimulation indices >2.0 in response to pooled normal IgG ($P < 0.001$), while all patients responded to an individual rheumatoid IgG with indices >2.8 ($P < 0.001$). Responses were dependent upon the dose of IgG used for stimulation and exhibited kinetics typical of antigen-primed lymphocytes, with maximal response on day 6.

In studies of thymidine incorporation by RA patients, one investigator has demonstrated responses to human IgG (13) while others have not (14–16). In experiments reported by Kinsella (13), aggregated IgG stimulated incorporation of [$^3$H]thymidine in unseparated peripheral blood leukocytes of 8 of 12 rheumatoid patients. Stimulation in these experiments was claimed to be dependent on the presence of complement and was maximal between days 5 and 6. Although no statistical differences between controls and RA patients could be demonstrated, the results were interpreted as indicating a positive correlation between RA and response to IgG because 67% of the patients exhibited stimulation indices >2.0. Reynolds and Abdou (14) reported similar experimental results in a system without complement, but interpreted the data negatively because no statistical differences were found between the responses of RA patients and controls. On the other hand, Kacaki et al. (15) and Runge and Mills (14) found no response to human IgG by peripheral blood leukocytes from RA patients.

Several considerations may account for the differences between the findings of other workers and the results presented here. As noted above, the peak of response after in vitro stimulation of lymphocytes from rheumatoid patients with human IgG occurs on day 6 with little response observable on day 4. Runge and Mills (16) reported only 4-d responses and their negative results could be interpreted on this basis. Other technical differences include concentration of responding cells, volume, level of stimulating antigen, and the fact that we used flat-bottomed microculture plates, whereas all other workers used macroculture conditions in test tubes. In addition, all previous workers examined the responses of unseparated peripheral blood leukocytes from rheumatoid patients. In our hands, isolated T cells from RA patients generally responded better than unseparated peripheral lymphocytes. This observation may indicate the presence of a suppressor cell population in unseparated cells that, like murine suppressor T cells, is removed by passage over nylon wool (17). Previous reports have established that although lymphocytes from rheumatoid synovial tissue lack suppressor cell activity, such activity is normal in peripheral blood of RA patients (18–20). Additional support for the concept that lymphocytes of RA patients recognize determinants on IgG comes from reports demonstrating that unseparated leukocyte preparations from RA patients produce migration inhibitory factor in response to either normal or aggregated IgG (21, 22). The possibility that migration inhibitory factor might be induced in response to light chain or Fab stimulation is presently under investigation.

The presence of T lymphocytes in RA patients responsive to human IgG may have significant implications for control of RF production in the rheumatoid patient. Indeed, recent studies indicate that B cells from RA patients or normal individuals responding in vitro to stimulation with PWM and producing RF recognize both helper and suppressor T cell signals (23–

TABLE II
*Stimulation of Peripheral T Cells from Controls and Rheumatoid Arthritis Patients with Subfractions of IgG*

| Group | Light chain preference | Stimulation index* | | | |
|---|---|---|---|---|---|
| | | Fab‡ | Fc | λ | κ |
| Controls | | | | | |
| J.M. | | 1.0 | 0.9 | — | — |
| M.L. | | 0.9 | 0.9 | — | — |
| C.E. | | 0.9 | 0.8 | — | — |
| E.D. | | 1.0 | 0.8 | — | — |
| R.T. | | 0.8 | 1.0 | — | — |
| RA patients | | | | | |
| C.G. | None | 3.6 | 1.4 | 5.2 | 3.9 |
| O.G. | | 4.0 | 1.0 | 4.0 | 3.2 |
| S.G. | | 5.9 | 1.1 | 2.3 | 2.6 |
| J.H. | | 2.8 | 1.8 | 2.8 | 2.8 |
| C.T. | | 6.4 | 2.1 | 5.1 | 4.9 |
| V.W. | λ | 3.6 | 1.9 | 4.1 | 1.4 |
| M.S. | | 3.0 | 1.1 | 5.2 | 1.3 |
| C.G. | | 4.1 | 2.1 | 6.7 | 1.8 |
| M.P. | | 3.2 | 1.7 | 3.9 | 1.9 |
| M.F. | κ | 3.5 | 1.1 | 1.7 | 5.7 |

* Counts per minute in cultures with antigen/counts per minute in cultures without antigen.
‡ $P < 0.001$ comparing controls with RA patients.

26). Although the functional role of the responding T cells described here has not been established, it is tempting to speculate that they provide helper activity for the production of RF. The vast majority of RF, however, recognize determinants within Fc (27). It is clear from the studies reported here that the antigenic stimulus responsible for the proliferative activity of T cells from patients with RA is located on Fab fragments and/or isolated light chains from human IgG. Essentially no T cell response was observed after culture with isolated Fc fragments. This dichotomy, however, is not surprising. In the mouse system, T and B cells responding to idiotypic determinants on isologous immunoglobulin molecules recognize separate antigenic sites. Thus, in the experiments of Jorgensen and Hannestad (28, 29), BALB/c helper T lymphocytes induced by the isologous mouse myeloma protein 315 (M315) recognize determinants within the light chain variable region. However, BALB/c anti-M315 idiotypic antibodies (and presumably the B cell receptor) recognize antigenic sites that are displayed only when the light and heavy chain variable regions are associated. In addition, in the immune response to lysozyme, T cells and B cells recognize different sequences on the lysozyme peptide chain (30). Thus, it appears that T cells and B cells need not recognize the same determinant for cooperative activity to occur. T cells from RA patients that respond to light chain and/or Fab determinants are, therefore, theoretically capable of cooperating with B cells recognizing Fc determinants. B cells that recognize Fc determinants on normal immunoglobulin molecules are presumably present in RA patients because most RF react with normal immunoglobulin Fc. In RA, and possibly particularly within the synovial cavity where suppressor function is minimal (18–20), such cooperative interactions may occur resulting in the production of autoreactive RF. In addition to RF, the production of antiidiotypic antibodies might also be expected. In fact, a recent study by Nasu et al. (31) reported detectable levels of antibodies with specificity for the Fd portion of IgG in 72% of patients with RA. Whether some of these anti-Fd antibodies are actually directed towards idiotypic determinants of Ig is not yet known.

Because autoreactive phenomena occur in RA, the rheumatoid patient represents a clinical model in which lymphocyte populations have been released from normal regulatory control. The inductive stimulus for this release, however, remains unknown. In this regard, it is noteworthy that T cells from certain individuals with RA respond preferentially to either lambda or kappa light chain determinants, whereas T cells from others respond essentially equally to both. Whether this finding has implications for etiology or pathogenesis, or simply represents expression of particular inherited immune response genes is not known. Because T cells comprise a heterogeneous population of cells, e.g., helper cells, suppressor cells, cytolytic cells, and cells involved in delayed hypersensitivity reactions, any or all of these cell types may be involved in the response to Fab and/or light chain determinants described here. The monoclonal antisera to human T cell subclasses that have recently become available should define the T cell type(s) involved.

Although responses were obtained with separate light chains as well as Fab the extent of overlap of these responses is not yet known. For example, responses to light chains may be directed solely towards "hidden determinants" (32, 33), whereas responses to Fab may be directed solely towards determinants present only on associated light and heavy chains. In this case, the responses to light chains would represent stimulation of clones of T cells quite different from those responding to Fab. Because IgM uses the same pool of light chains as IgG, the inefficiency of IgM as a stimulating agent (only 3 of 26 patients responded) might argue in favor of hidden or associated sites as the relevant determinants. However, basic conformational differences between IgM and IgG might also account for the different responses. It is unlikely that the small number of patients reactive to IgM were due to contamination with IgG because we calculate that such contamination must be <5%, i.e., 250 $\mu$g of IgM could contain no more than 12.5 $\mu$g of IgG. This amount is well below the amount of IgG inducing significant incorporation of thymidine (Fig. 3). Furthermore, the three patients responding to IgM were only average responders to IgG. If contamination with IgG caused responses, one would expect high responders to IgG to also respond to IgM.

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