Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 549–553, January 1992
Medical Sciences

Perforin and granzyme A expression identifying cytolytic lymphocytes in rheumatoid arthritis

GILLIAN M. GRIFFITHS*†‡, SUSAN ALPERT*†, ELAINE LAMBERT§, JAMES MCGUIRE§, AND IRVING L. WEISSMAN*†¶

*Howard Hughes Medical Institute and Departments of †Pathology and §Medicine, Stanford University, Stanford, CA 94305

Contributed by Irving L. Weissman, September 16, 1991

ABSTRACT **Lymphocytes from the synovial fluid of patients with rheumatoid arthritis were examined for the expression of granzyme A and perforin. Previous studies have demonstrated that the expression of these proteins, which are implicated as mediators of cytotoxicity, can be used to identify putative cytolytic lymphocytes *in vivo*. Twenty-two synovial fluid samples were analyzed by *in situ* hybridization and immunohistochemistry. In six patients receiving low doses of immunosuppressant, a population of granzyme A- and perforin-expressing lymphocytes could be identified. In contrast, lymphocytes from patients who were receiving high doses of immunosuppressant did not contain any granzyme A- or perforin-expressing lymphocytes. Synovial fluid lymphocytes from patients with osteoarthritis did not express either marker. The expression of these markers demonstrates the presence of potentially functional cytolytic lymphocytes, expressing proteins required to mediate killing, in the synovial fluid of patients with rheumatoid arthritis. This suggests that cytolytic lymphocytes may be involved in the pathogenesis of rheumatoid arthritis.**

Rheumatoid arthritis (RA) is a chronic inflammatory disease of the joints, characterized by lymphocytic infiltration of the synovial membrane and eventual destruction of the cartilage and bone in affected joints. The causative agents that initiate the disease, and the underlying mechanisms that result in damage, remain largely obscure. The HLA linkage and the autoantibody production observed in a majority of patients support an autoimmune element in this disease (1–4). The relative contributions of the different lymphocytes to the disease is difficult to determine. The synovial fluid (SF) contains neutrophils and macrophages as well as T and B lymphocytes. With the demonstration that rheumatoid factors were anti-immunoglobulin antibodies that could fix complement, early studies on the immunopathogenesis of the joint lesions favored a primary role for the immune complexes, complement, and neutrophils (5, 6).

Several studies have suggested that synovial T cells are activated by virtue of elevated levels of HLA-DR molecules and mRNA encoding lymphokines (7–10). Many studies have used CD4 and CD8 cell-surface markers to characterize the function of these lymphocytes. The ratio of CD4 to CD8 lymphocytes varies within the joint (11, 12). In general, a predominance of CD4 cells has been reported (13, 14). Since CD4 is usually associated with lymphocytes displaying "helper cell" activity, this has led to the suggestion that the role of these cells is predominantly a regulatory one in order to stimulate B cells to produce antibody. However, the expression of CD4 only indicates that antigen will be seen associated with major histocompatibility complex (MHC) class II molecules on antigen presenting cells and does not necessarily denote function. In fact, both CD4 and CD8 cells can be cytolytic, and this would suggest a very different role for the T cells found in the RA joints.

To test the hypothesis that cytolytic T cells (of either the CD4 or CD8 phenotype) play a role in RA, we have used markers that correlate with the function of cytolytic lymphocytes—granzyme A and perforin. These proteins appear to be involved in the killing process as they are expressed only in activated killer cells and their expression appears to be tightly regulated (15–19). It has been proposed that a function of released granzyme A is to induce endonucleolytic degradation of target cell DNA (20). Perforin is itself a cytolytic molecule able to form pores in the membranes of target cells (21). Consequently, it has been possible to use these molecules as markers for cytolytic lymphocyte responses *in vivo* (16, 22–25).

By *in situ* hybridization, we have been able to identify cytolytic lymphocytes expressing granzyme A and perforin in the SF of patients with inflammatory RA. These markers identified activated cytotoxic lymphocytes only in patients with severe RA who were not being treated with high levels of immunosuppressive agents. SF of RA patients receiving high doses of immunosuppressive agents lacked these markers, as did SF from patients with noninflammatory arthritides (osteoarthritis). These data support a possible role for cytolytic lymphocytes in the pathogenesis of RA.

METHODS

Sample Preparation. Twenty-two SF samples from patients with well established arthritis were tapped during routine clinical treatment for symptomatic inflammatory arthritis. All patients had been diagnosed as having class II inflammatory SF. All patients had cell counts of >5000 cells per cm^3. Samples were placed in heparinized tubes before washing and pelleting the cells in RPMI 1640 medium. Cells were then resuspended in medium and cells were cytocentrifuged onto poly-(L-lysine)-coated slides (4×10^5 cells per slide). Samples were either fixed in 4% paraformaldehyde and dehydrated through a graded series of alcohols and stored for *in situ* hybridization at 4°C or were fixed in ice-cold acetone for 10 min and stored at −70°C for subsequent immunohistochemical analysis.

Preparation of Labeled Probes. The full-length 950-base-pair (bp) cDNA of human granzyme A (previously termed HF) (15) and two cDNA fragments of the human perforin gene (19) corresponding to either 1100 bp of the 5′ sequence or 1400 bp of the 3′ sequence were cloned into the pSPT673 vector containing SP6 and T7 promoters. After linearization of the vector by using appropriate restriction enzymes, sense and antisense RNA probes were prepared with SP6 and T7

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: RA, rheumatoid arthritis; MHC, major histocompatibility complex; SF, synovial fluid.

‡Present address: Basel Institute for Immunology, Basel, CH 4005 Switzerland.
¶To whom reprint requests should be addressed.

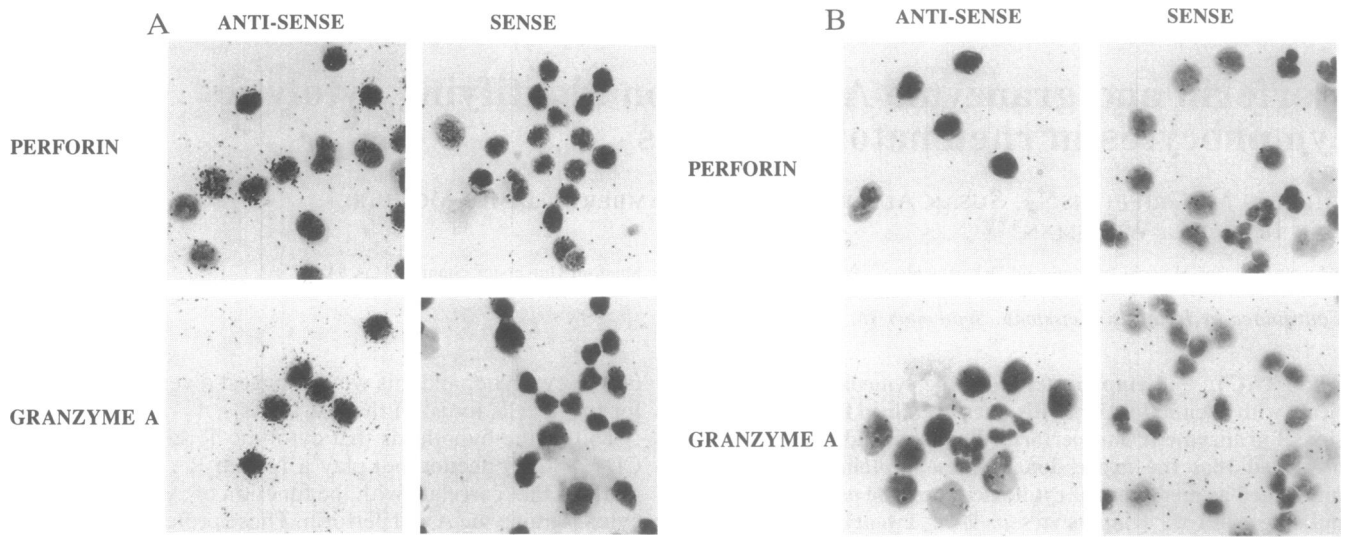

FIG. 1. *In situ* hybridization using sense or antisense probes for granzyme A and perforin on SF lymphocytes. SF cells from a patient with RA and no immunosuppressive treatment (patient 1) (*A*) or immunosuppressive treatment (patient 8) (*B*).

polymerases (Promega). The preparation of the probe was as described (22).

***In Situ* Hybridization and Evaluation of Slides.** Paraformaldehyde-fixed cells were treated with proteinase K (Sigma) at 1 μg/ml in 100 mM Tris·HCl, pH 8.0/50 mM EDTA at 37°C for 30 min and fixed again in paraformaldehyde as described above. Slides were then acetylated by treatment with 0.25% acetic anhydride in 0.1 M triethanolamine for 10 min. Hybridizations were carried out in 10 μl of hybridization mixture (50% formamide/10% dextran sulfate/100 mM dithiothreitol/300 mM NaCl/20 mM Tris·HCl, pH 7.5/5 mM EDTA/1× Denhardt's solution), using 2×10^6 cpm/μl. Hybridization was carried out for 12–16 hr at 50°C. Slides were then washed in 50% formamide/2× standard saline citrate/20 mM Tris·HCl, pH 7.5/5 mM EDTA. Four washes of 30 min each were carried out at 50°C. Hybridized slides were then treated with RNase A (20 μg/ml) and RNase T1 (1 unit/ml) for 30 min at 37°C. Slides were dipped in 50% NTB2 nuclear track emulsion (Kodak) containing 300 mM ammonium acetate and were exposed for 14 and 21 days at 4°C. Slides were developed with Kodak developer D19 for 2.5 min and Kodak fixer for 5 min. Cells were immediately counterstained with 4% Giemsa stain for 10 min.

Immunohistochemistry. Immunohistochemical staining was performed on acetone-fixed samples, which were incubated with primary antibody for 30 min and then sequentially with rabbit anti-mouse IgG (Dako, Carpinteria, CA), swine anti-rabbit IgG (Dako), and then rabbit peroxidase-antiperoxidase (Dako) for 30 min each and reacted using 3,3′-diaminobenzidine (Sigma) as substrate. Slides were then counterstained with hematoxylin, dehydrated through a graded series of alcohols, and mounted. The following monoclonal antibodies were used for the first stage: Leu2a (anti-CD8; Becton Dickinson) and Leu3a+b (anti-CD4; Becton Dickinson).

Rheumatoid Factor Screening. Samples were screened by latex agglutination. Serial dilutions of the samples were incubated with latex beads (Wampole Labs, Cranburg, NJ) at 37°C for 15 min; the particles were then pelleted and subsequently resuspended. The presence of agglutinated latex beads was assessed as described (27).

RESULTS

Granzyme A and Perforin Expression in SF Lymphocytes from Patients with RA. Thirteen SF samples from 10 patients with inflammatory RA were examined for the presence of lymphocytes expressing granzyme A and perforin by *in situ* hybridization. Six patients, who were receiving no or very low doses of immunosuppressant, were found to contain lymphocytes expressing granzyme A and perforin (Fig. 1*A*). Quantitative analyses were carried out by counting a minimum of 300 lymphocytes; between 2% and 75% of SF lymphocytes were positive with either the perforin or the granzyme A probe (Table 1). The number of cells expressing granzyme A was always approximately the same as the number of cells expressing perforin, supporting previous observations that it is the same subset of lymphocytes expressing both proteins.

Patients 1–6 (Table 1) were being treated with either no steroids or low doses of steroids at the time that these samples were obtained. Patients 2 and 3 were receiving low doses of steroids prior to total lymphoid irradiation in an attempt to ameliorate the disease after it had failed to respond

Table 1. SF lymphocytes from untreated RA patients express granzyme A and perforin

Patient	Prednisone, mg/day	Methotrexate, mg/day	Cell count	% polymorphonuclear cells	CD4/CD8 ratio	% granzyme A positive	% perforin positive
1	5	0	14,000	85	1:1	63	66
2	7.5	0	10,000	75	1:1	30	30
3	10	0	60,000	40	1:1	75	74
4	0	0	18,000	85	2:1	60	ND
5	5	7.5	35,000	80	4:1	30	30
6	0	7.5	7,000	70	1:1	2	2

Percentages of lymphocytes expressing granzyme A and perforin in SF lymphocytes from patients with RA. Quantitative analyses were carried out by counting a minimum of 300 lymphocytes. Cells were counted as positive when the number of grains above them was >5-fold background. ND, not determined.

Table 2. SF lymphocytes from RA patients receiving immunosuppressive treatment do not express granzyme A and perforin

Patient	Prednisone, mg/day	Other treatment	Cell count	% polymorphonuclear cells	CD4/CD8 ratio	% granzyme A positive	% perforin positive
7	None	None	25,000	90	ND	0	0
8	30	None	60,000	95	ND	0	0
8	8	None	50,000	90	ND	0	0
9	4	Pen	10,000	70	2:1	0	0
10	5	Pen	10,000	40	1:1	0	0
3*	None	TLI	50,000	90	90:1	0	0
5*	40	Methotrexate (20 mg/wk)	65,000	75	ND	0	0

In situ hybridization using antisense and sense probes were carried out on cytospin slides with $\approx 4 \times 10^5$ cells per slide. No positively hybridizing cells were detected. All experiments were carried out with a positive control of the human cytotoxic cell line Aα JY, where positively hybridizing cells were always observed with the antisense probe and never with the sense probe. Two samples from patient 8 were obtained over a 5-month interval, during which time the dose of prednisone had been reduced. Patients 9 and 10 were receiving penicillamine (pen). ND, not determined.
*Samples from patients also shown in Table 1 after total lymphoid irradiation (TLI) or methotrexate treatment.

to other drugs. Patient 4 had not been treated for 2 years and the other patients were receiving low doses of steroids as described in Table 1.

Table 2 shows the results obtained from seven other SF samples from RA patients in which no granzyme A- or perforin-expressing lymphocytes were detected. All except one of these samples were obtained from patients receiving high doses of glucocorticoids or other treatments. None of these contained any lymphocytes expressing granzyme A or perforin (Fig. 1*B*). Patient 8 had been maintained on 30 mg of prednisone per day at the time of the first analysis. A second sample, obtained 4 months later when the dose had been dropped to 8 mg/day, showed no change in the synovial lymphocyte population. Patients 9 and 10 were receiving penicillamine treatment. One patient who had received no form of treatment (Table 2, patient 7) showed no granzyme A-positive/perforin-positive lymphocytes. It should be noted that this sample was taken at the time of first diagnosis of the disease.

In two cases (Tables 1 and 2; patients 3 and 5) it was possible to examine the SF lymphocyte population of previously positive patients after treatment. In both instances, the number of lymphocytes expressing granzyme A and perforin was reduced to zero after treatment (Table 2). Patient 3 received total lymphoid irradiation treatment in the intervening time between the samples. Patient 5 had been treated with the higher doses of steroids for the 5 months between samples. In addition, this patient had also received an intraarticular injection of steroids at the time of the earlier visit. This supports the observed correlation between the immunosuppressive therapy and the lack of granzyme A- and perforin-expressing lymphocytes.

Table 3. Presence of rheumatoid factor and duration of disease in RA patients

Patient	Rheumatoid factor	Duration, years
1	Negative	2
2	Positive	7
3	Positive	10
4	Negative	2 (no treatment)
5	Positive	7
6	Positive	3
7	Positive	First diagnosis
8	Negative	16
9	Positive	3
10	Positive	12

Samples were assessed for the presence of rheumatoid factor (RF) as described. The titers at which latex agglutination was determined are given. Patients with titers of <1:320 were considered negative for RF. Patient 4 had received no treatment for the 2-year period since the initial diagnosis. Patient 7 was diagnosed as having RA at the time the SF sample was obtained.

In Table 3 the incidence of rheumatoid factor was tested in the same patients. There was no correlation between the expression of granzyme A and perforin and the presence of rheumatoid factor. Nor was there an obvious correlation with the length of duration of the disease.

Phenotypic Analysis of SF Lymphocytes. To determine whether CD4 or CD8 lymphocytes were expressing granzyme A and perforin, the SF samples were stained by immunohistochemistry to determine the CD4/CD8 ratios, as shown in Tables 1 and 2. These ratios varied, although in every case at least 50% of the cells were of the CD4 phenotype. Since >50% of lymphocytes from patients 1, 3, and 4 were expressing granzyme A and perforin, it follows that both $CD4^+$ as well as $CD8^+$ lymphocytes must be expressing granzyme A and perforin. Therefore, $CD4^+$ cells in the joint can express markers associated with functionally cytolytic lymphocytes.

Granzyme A- and Perforin-Expressing Lymphocytes in Osteoarthritis. SF infiltrates from five patients with osteoarthritis were also examined for the presence of lymphocytes expressing granzyme A and perforin (Table 4). The patients from whom these samples were obtained were negative for rheumatoid factor and were not receiving immunosuppressive drugs. However, in marked contrast to the samples obtained from the rheumatoid patients, none of the infiltrating lymphocytes was found to be expressing granzyme A or perforin.

Granzyme A and Perforin Expression in HLA-B27-Linked Arthritis. A small number of samples were obtained from patients suffering from a number of other types of arthritis

Table 4. SF lymphocytes from patients with osteoarthritis do not express granzyme A and perforin

Patient	% granzyme A positive	% perforin positive
1	0	0
2	0	0
3	0	0
4	0	0
5	0	0

Approximately 4×10^5 cells were analyzed by *in situ* hybridization. No hybridizing cells were detected. All experiments were carried out with the cytotoxic line Aα JY as a positive control.

Table 5. Percentages of lymphocytes expressing granzyme A and perforin in HLA-B27-related arthropathies

Arthritides	% granzyme A positive	% perforin positive
Reiter syndrome	0	0
Psoriatic arthritis	0	0
Crohn disease	0	0
Ankylosing spondylitis	80	80

Single samples from patients were analyzed by using $\approx 4 \times 10^5$ cells for *in situ* hybridization. The results were quantitated by counting a minimum of 300 lymphocytes.

that appear to be prevalent in people with the HLA-B27 haplotype (Table 5).

Single samples obtained from patients suffering from Reiter syndrome, psoriatic arthritis, and Crohn disease did not contain any granzyme A- or perforin-expressing lymphocytes. However, 80% of the SF lymphocytes from a patient with ankylosing spondylitis were expressing granzyme A and perforin.

DISCUSSION

The findings presented here raise three important points. First, they provide evidence that lymphocytes within RA joints display the cytolytic phenotype, suggesting that cytotoxic lymphocytes may contribute to the pathogenesis of the disease. Second, the samples analyzed suggest that immunosuppressive treatments that ameliorate the disease are able to suppress selectively the appearance of these cytolytic lymphocytes. Third, these findings provide evidence, albeit indirect, of the generation of cytolytic CD4 lymphocytes *in vivo*.

This study demonstrates the presence of lymphocytes expressing granzyme A and perforin in the SF of RA patients. The significance of this finding stems from what is known about the expression and function of these markers. In experimentally controlled studies in mice these proteins, and the genes that encode them, are only expressed in activated cytolytic lymphocytes (22). Furthermore, these proteins appear to be functionally involved in the cytolytic process whereby T lymphocytes and natural killer cells can directly destroy the target cells that they recognize. Granzyme A has been reported to be responsible for inducing target cell DNA degradation (20), while perforin forms multimers in the plane of the cell membrane that are 20-nm pores (21). Therefore, granzyme A and perforin expression provide markers for putative functional cytolytic lymphocytes.

One important aspect of this study is that we obtained SF samples from patients with well established RA who were receiving little or no immunosuppressive drugs. Only in these patients were granzyme A- and perforin-expressing lymphocytes observed. Thus, the presence of granzyme A and perforin in the untreated patients argues in favor of these cells being functionally cytolytic lymphocytes within the RA joints.

In the two instances in which it was possible to obtain samples from the same patients both before and after treatment, the disappearance of granzyme A- and perforin-expressing lymphocytes after treatment was striking. Both total lymphoid irradiation and an increase in immunosuppressive drugs produce this effect. The fact that lymphocytes in the joints of four other patients receiving high levels of immunosuppressive drugs also failed to express granzyme A and perforin suggests that these drugs may improve the disease by suppressing cells that express granzyme A and perforin and hence decreasing the cytolytic capability of lymphocytes within the joints.

For comparative purposes, we also analyzed samples from patients with osteoarthritis. One way in which osteoarthritis differs in its pathology from RA is that it is associated with only minor degrees of inflammation. These SF samples did not contain any granzyme A- or perforin-expressing lymphocytes, demonstrating that lymphocytes that have migrated to the site of this type of inflammation need not necessarily be activated.

Although the presence of T lymphocytes in both the SF and the synovium has been well documented, the function of these cells is still unclear. Previous studies have been able to demonstrate that many of the T lymphocytes within RA joints are activated by virtue of expression of MHC class II antigens and interleukin 2 receptor (3–6). However, these studies do not address the potential functional activity of these T cells. Both helper and cytolytic lymphocytes express interleukin 2 receptors when activated. Most clinical studies have used the CD4 and CD8 phenotypes as indicators of function, interpreting the CD4 cells to be helper cells and CD8 to be cytotoxic/suppressor cells. However, CD4 and CD8 are MHC co-receptors; CD8 T cells recognize MHC class I molecules bearing antigen peptides, whereas CD4 cells recognize MHC class II molecules bearing antigen peptides. It seems likely that most cytolytic T cells are $CD8^+$ because most somatic cell targets (e.g., virally infected cells) are MHC class I^+ class II^- and that most helper T cells are $CD4^+$ because MHC class II is expressed at very high levels by the cells with which helper T cells interact—B cells and antigen presenting macrophages and dendritic cells. This correlation is deceptive and could blind the observer to important departures from "conventional wisdom." CD4 killer T cells exist and obviously must find MHC class II^+ targets. Inflammatory cytokines such as interferon γ may induce high levels of MHC class II on a number of somatic cell types, including inflamed synovium (28–30). Here we demonstrate the presence of both CD4 and CD8 T cells expressing functional markers of cytolytic cells. It would be surprising if they did not find and destroy MHC class I and class II targets in the synovium.

The fact that the disease involves inflammatory processes implies, but does not prove, that a local antigen-specific immune response plays a crucial role in disease pathogenesis. It has been proposed that mycobacterial antigens are important in RA since mycobacterial reactive clones have been isolated from synovia (31). However, synovial T cells do not always appear to be clonally restricted in their T-cell receptor usage and this argues against a single local antigen response (32–37). Lymphocytes can also reach inflammatory sites in other ways: by recognition of inflammatory endothelium (26) or by chemotaxis toward factors released by activated cells. The experiments presented here do not address the questions of whether a specific antigen response is involved or whether activation is local or distant. What is clear from these experiments is that putative cytolytic lymphocytes, capable of mediating target cell destruction, are present at the site of inflammation.

It seems likely that several different mechanisms are involved in the pathogenesis of RA, and it is possible that different mechanisms predominate at different phases of the disease. Exactly when the cytolytic lymphocytes identified in this study play an important role remains to be discovered. The single case of a newly diagnosed patient (Table 2, patient 7) in which the cytolytic phenotype was not apparent suggests that the cytolytic lymphocytes are not the primary pathogen in the disease. However, the presence of cytolytic lymphocytes in patients at later stages of the disease and the fact that immunosuppressive treatments that improve the disease also selectively remove the cytolytic phenotype from the site of inflammation supports an important role for these cells at some stage of the disease.

This study suggests that cytolytic lymphocytes may play a role in the pathogenesis of RA. In this study, we demonstrate that both CD4 and CD8 lymphocytes may be involved in a cytolytic response, and this suggests that the antigen(s) seen is recognized in the context of both class I and class II MHC molecules. How these cells reach the synovium and whether there is a local antigen is not addressed by this study. We only suggest that a cytolytic T-cell response might be involved in the pathogenesis of the disease.

We would like to thank Dr. Reiko Namikawa for continuous support and encouragement throughout this work, Dr. Carol Clayberger for Aα JY cells, Drs. C.-C. Liu and Ding-E. Young for perforin cDNA, and Drs. Garry Fathman and Alexandra Livingstone for critical reading of the manuscript. This work was funded by grants from the Howard Hughes Institute and National Institutes of Health Grant 5PO1 AI-19512 (to I.L.W.). G.M.G. was the recipient of a postdoctoral fellowship from the Arthritis Foundation. S.A. was the recipient of a National Institutes of Health postdoctoral fellowship (CA 08590-03).

1. Stastny, P. (1978) *N. Engl. J. Med.* **298,** 869–871.
2. Harris, E. D. (1990) *N. Engl. J. Med.* **322,** 1277–1289.
3. Ziff, M. (1990) *J. Rheumatol.* **17,** 127–133.
4. Kingsley, G., Pitzalis, C. & Panayi, G. S. (1990) *Br. J. Rheumatol.* **29,** 58–64.
5. Carson, D. A. (1985) in *Textbook of Rheumatology*, eds. Kelly, W. J., Harris, E. D., Jr., Ruddy, S. & Sledge, C. B. (Saunders, Philadelphia), p. 664.
6. Winchester, R. J., Agnello, V. & Kunkel, M. J. (1970) *Clin. Exp. Immunol.* **6,** 689–706.
7. Klareskog, L. & Johneu, O. (1988) *Br. J. Rheumatol.* **27,** Suppl. 2, 141–142.
8. Cush, J. J. & Lipsky, P. E. (1988) *Arthritis Rheum.* **31,** 1230–1238.
9. Firestein, G. S., Alvaro-Garcia, J. M. & Maki, R. (1990) *J. Immunol.* **144,** 3347–3353.
10. Nykanen, P., Bergroth, V., Ramnio, P., Nordstrom, D. & Kontinen, Y. T. (1986) *Rheumatol. Int.* **6,** 269–271.
11. Kurosaka, M. & Ziff, M. (1983) *J. Exp. Med.* **158,** 1191–1210.
12. Fox, R. I., Fong, S., Sabharwal, N., Carstens, S. A., Kung, P. C. & Vaughn, J. H. (1982) *J. Immunol.* **128,** 351–354.
13. Lasky, H. P., Bauer, K. & Pope, R. M. (1988) *Arthritis Rheum.* **31,** 52–59.
14. Kingsley, G., Pitzalis, C., Kyriazis, N. & Parayi, G. S. (1988) *Scand. J. Immunol.* **28,** 225–232.
15. Gershenfeld, H. K., Hershberger, R. J., Shows, T. B. & Weissman, I. L. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 1184–1188.
16. Hershberger, R. J., Mueller, C., Gershenfeld, H. K. & Weissman, I. L. (1989) *Curr. Top. Microbiol. Immunol.* **140,** 81–92.
17. Jenne, D. E. & Tschopp, J. (1988) *Curr. Top. Microbiol. Immunol.* **140,** 33–48.
18. Bleackley, R. C., Lobe, C. G., Duggan, B., Ehrman, N., Fregeau, C., Meier, M., Letellier, M., Havele, C., Shaw, J. & Paetkau, V. (1988) *Immunol. Rev.* **103,** 5–20.
19. Lui, C.-C., Rafii, S., Granelli-Piperno, A., Trapani, J. A. & Young, J. D.-E. (1989) *J. Exp. Med.* **170,** 2105–2118.
20. Hayes, M. P., Berresi, G. A. & Henkart, P. (1989) *J. Exp. Med.* **170,** 933–946.
21. Podack, E. R., Olson, K. J., Lowrey, D. M. & Lichtenheld, M. G. (1989) *Curr. Top. Microbiol. Immunol.* **140,** 11–18.
22. Mueller, C., Gershenfeld, H. K., Lobe, C. G., Okada, C. Y., Bleackley, R. C. & Weissman, I. L. (1988) *J. Exp. Med.* **167,** 1124–1136.
23. Mueller, C., Kägi, D., Aebischer, T., Odermatt, B., Held, W., Podack, E. R., Zinkernagel, R. M. & Hengartner, H. (1989) *Eur. J. Immunol.* **19,** 1253–1259.
24. Wood, G. S., Mueller, C., Warnke, R. A. & Weissman, I. L. (1988) *Am. J. Pathol.* **133,** 218–225.
25. Griffiths, G. M., Namikawa, R., Mueller, C., Liu, C.-C., Young, J. D.-E., Billingham, M. & Weissman, I. L. (1991) *Eur. J. Immunol.* **21,** 687–692.
26. Jalkanen, S., Steere, A. C., Fox, R. I. & Butcher, E. C. (1986) *Science* **233,** 556–558.
27. Ritzman, S. & Daniels, J. (1981) in *Serum Protein Abnormalities: Diagnostic and Clinical* (Little, Brown, Boston), p. 318.
28. Alvaro-Gracia, J. M., Zvaifler, N. J. & Firestein, G. S. (1989) *J. Exp. Med.* **170,** 865–876.
29. Amento, E. P., Bhan, A. K., McCullagh, K. G. & Krane, S. M. (1985) *J. Clin. Invest.* **76,** 837–848.
30. Burmester, G. R., Jahn, B., Rowher, B., Zacher, J., Winchester, R. J. & Kalden, J. R. (1987) *J. Clin. Invest.* **80,** 595–604.
31. Holoshitz, J., Koning, J., Coligan, J. E., De Bruyn, J. & Strober, S. (1989) *Nature (London)* **339,** 226–229.
32. Hill Gaston, J. S., Life, P. F., Bailey, L. C. & Bacon, P. A. (1989) *J. Immunol.* **143,** 2494–2500.
33. Stamenkovic, I., Stegagno, M., Wright, K. A., Krane, S. M., Amento, E. P., Colvin, R. B., Duquesnoy, R. J. & Kurnick, J. T. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 1179–1183.
34. Duby, A. D., Sinclair, A. K., Osborne-Lawrence, S. L., Zeldes, W., Kan, L. & Fox, D. A. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 6206–6210.
35. Brennan, F. M., Allard, S., Londei, M., Savill, C., Boylston, A., Carrel, S., Maini, R. N. & Feldmann, M. (1988) *Clin. Exp. Immunol.* **73,** 417–423.
36. Savill, C. M., Delves, P. J., Kioussis, D., Walker, P., Lydyard, P. M., Colaco, B., Shipley, M. & Roitt, I. M. (1987) *Scand. J. Immunol.* **25,** 629–635.
37. Keystone, E. C., Minden, M., Klock, R., Poplonski, L., Zalcberg, J., Takadera, T. & Mak, T. W. (1988) *Arthritis Rheum.* **31,** 1555–1557.