Commentary

When Do Microbes Stimulate Rheumatoid Factor?

By David N. Posnett and James Edinger

From the Immunology Program, Laboratory of Human Molecular Immunobiology, and the Department of Medicine, Cornell University Medical College, New York 10021

Anti–γ-globulins or rheumatoid factors (RF) were first described in the early 1940s. They are present in >70% of patients with rheumatoid arthritis (RA) and high titers are associated with severe disease, but they also appear in a large number of other diseases including viral, bacterial, and parasitic infections (1). In spite of their wide spread occurrence, it is still a puzzle how or why RF are generated.

RF come in two varieties. Low affinity RF (K_d of $\sim 10^{-5}$ M) are IgM natural antibodies with specificity for IgG-Fc determinants and cross-reactivity with other autoantigens, i.e., polyreactivity. They are produced by $CD5^+$ B cells in normal subjects (2). Frequently they are IgM κ antibodies and use selected germline V genes for both H and L chains. This is why they share cross-reactive idiotypes, as discovered by Kunkel et al. in the 1970s (3). These antibodies are typically T cell independent. They are similar to the RF produced in response to polyclonal B cell activation by EBV (4) or LPS (5, 6). B cells producing these RF appear to be susceptible to malignant transformation as the RF-associated V genes are frequently expressed in low grade chronic B lymphoproliferative diseases such as chronic lymphocytic leukemia, Waldenstrom's macroglobulinemia, mixed cryoglobulinemia and lymphoma associated with Sjögren's disease. This may be due to constitutive expression of STAT3 in B1 cells (7). The RF-associated V genes are also over-expressed by human fetal B cells (8, 9), perhaps consistent with a role for low affinity RF in neonates that lack a mature humoral immune system. In spite of the low affinity, the multivalency of IgM RF allows excellent agglutination of latex particles or red blood cells, and presumably also microbial organisms, in vivo, that are coated with specific IgG antibodies. The presence of IgM RF can lead to large immune complexes with lattice formation, that are poorly soluble and rapidly removed by the mononuclear phagocyte system (10).

High affinity RF (K_d of $\sim 10^{-7}$ M) can be IgM, IgG, IgA, or IgE antibodies. RA patients may have high titers of this type of RF. Their production is T cell dependent. These antibodies often do not share the V genes used by the low affinity RF (11). They have undergone affinity maturation, as there are multiple somatic mutations in the V genes, and are therefore produced by antigen driven B cells. These RF bind most avidly to the Ig isotype which stimulated their production. In RA, RF are particularly abundant in the synovium. In some reports the dominant specificity of synovial RF is for IgG3-Fc (12), implying that the immune complexes that stimulated their local production contained IgG3.

Experimental production of RF has been described with either polyclonal B cell stimulation or immunizations with immune complexes. In mice treated with LPS, high titers of T-independent RF are produced (5, 6). In humans, EBV is a polyclonal B cell activator, and in vitro transformation with EBV results in the production of IgM RF (2, 4). In contrast, immunizations with immune complexes result in T-dependent high-affinity RF. Thus, a mixture of a complex multivalent antigen, *Limulus polyphemus* hemocyanin conjugated with a hapten (*p*-azophenylarsonate), and IgG1 antibody to the hapten, results in IgM anti-IgG1-Fc RF. In addition, when an IgG2a antibody specific for the hapten has been used, the IgM RF is specific for IgG2a-Fc (13). The RF response does not occur in nude mice and is therefore T-dependent, unlike the RF induced by LPS. Similar results are obtained with TNP-conjugated transferrin or KLH, which are also multivalent antigens, and the importance of saturation of the immune complex with antibody has been shown, as well as the requirement for primed T cells (14). Relevant to RA, the multivalent antigen, collagen type II, complexed with a monoclonal anti-collagen II IgG2a antibody, induces IgG anti-IgG-Fc RF in mice (15). One such RF, immortalized as a hybridoma, has unusual dual reactivity as an RF and an antiidiotype against the anticollagen mAb used for immunization. It is common to see antiidiotypic antibodies and high-affinity RF in secondary immune responses. Both represent antibodies against a self antibody and are thought to play a role in regulation of the immune response, but they may also participate in clearance of immune complexes (1).

In this issue of *The Journal of Experimental Medicine*, Fehr et al. describe a novel way to generate antibodies against antibodies (16). They use UV-inactivated vesicular stomatitis virus (VSV). The envelope of this virus is densely packed with the single viral glycoprotein, VSV-G (17). The antigenic epitopes are regularly spaced, 5–10 nM apart, in this rigid paracrystalline membrane (18). The authors have previously suggested that some viruses like VSV with structurally rigid envelopes, unlike the fluid membranes of eukaryotic cells where membrane proteins have lateral mobility, are associated with production of T cell–independent antibodies (18). To induce antiantibodies, they coat VSV with saturating amounts of monoclonal IgM anti-VSV-G antibodies and immunize BALB/c mice twice over 14 d. IgG anti-

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/97/05/1721/03 $2.00
Volume 185, Number 10, May 19, 1997 1721–1723

IgM antibody was detected at serum titers up to 1:20,480. These antiimmunoglobulins were antiidiotypic. They were not elicited if IgG anti-VSV antibodies were used instead of the IgM, nor if recombinant, soluble VSV-G protein was expressed as micelles and then complexed with IgM anti-VSV-G, suggesting a requirement for a high concentration of VSV-G in the rigid paracrystalline envelope of VSV. IgM anti-VSV-G complexed using a rat anti-κ antibody also failed to induce antiimmunoglobulins. In A/J mice of IgM^e haplotype immunized in the same way, the antiimmunoglobulins were allotypic as the immunizing IgM anti-VSV-G is of BALB/c origin (IgM^a). In both systems, these IgG antiimmunoglobulins could not be raised in CD4-depleted or nude mice, i.e., they were T-dependent responses.

Similar immunizations were performed with UV-inactivated bacteria coated with anti-LPS IgG2a or IgG2b antibodies specific for the highly repetitive *O*-linked external sugar chains of LPS which carry the immunogenic epitopes. Because LPS is also tightly packed on the outer cell wall of bacteria, this is considered a rigid structure. *Pseudomonas aeruginosa* coated with syngeneic IgG2b anti-LPS stimulated the production of IgG anti-IgG2b RF. These were not seen in LPS nonresponder C3H/HeJ mice, but bacteria alone, without complexed anti-LPS antibody, did not stimulate RF production. Whether this was a T cell–dependent response was not tested. Finally, *Salmonella typhi* coated with IgG2a anti-LPS (of NZB origin) stimulated production of allospecific IgG anti-IgG recognizing only NZB IgGs.

These data provide a direct link between exposure to intact virus or bacteria and the development of antiimmunoglobulins. They suggest that the occurrence of antiimmunoglobulins in vivo may be related to chronic exposure to microbes that are particularly immunogenic due to the high concentration of epitopes on their surface. In the case of a low grade chronically productive infection (HIV, herpesviruses, untreated bacterial endocarditis), the microbes would be complexed with host antibodies, and this apparently provides an excellent stimulus for the production of anti-γ-globulins.

The rules for RF production by complexed viruses are clearly not the same as those for T-independent responses (18). RF were induced only in the *Pseudomonas* model by Fehr et al. (16). Polyclonal B cell stimulation by LPS was required. In addition, stimulation with the complexed IgG2b anti-LPS antibody was also necessary. This is of interest because it raises the question of whether both antigenic stimulation with immune complex and nonspecific B cell stimulation are required for the generation of RF. Earlier results may be consistent with this idea because polyclonal B cell stimulation with LPS (5, 6) could induce autoantibodies to multimeric autoantigens or latent viruses such that unintended immune complexes would develop. With EBV-stimulated B cells (4), the viral envelope itself could be complexed with antibodies.

Two classical clinical diseases associated with high titer RF are subacute endocarditis and RA. In subacute bacterial endocarditis the culprit is frequently a *Streptococcus*. Once the offending agent is removed with successful antibiotic therapy, the RF disappear (19). In RA, the nature of the stimulus for high titer RF is unknown, but several viruses have been suggested in the etiology of RA including rubella, lentivirus, parvovirus, and EBV. The original excitement about EBV was due to the description of antibodies, in sera from RA and Sjögren's syndrome patients, that were reactive with EBV-transformed B cell lines (20). Recently the interest in EBV has been revived. When compared with controls, RA patients may have higher levels of EBV shedding in the throat, increased percentages of infected B cells in the blood, and higher levels of antibodies to EBV according to some reports. IgG anti-VCA titers to EBV may correlate with high titers of IgM RF in RA (21). Reactivation of latent EBV may be associated with a dominant IgG3 anti-VCA response (22). Could this be related to the suggested isotype specificity of synovial RF in RA? In four out of nine RA patients, a significant proportion of synovial fluid CD8 T cells were reactive with HLA-A2–restricted peptides derived from BMLF1 or BZLF1 (23, 24), early transactivators of EBV that initiate lytic phase viral replication. Serum antibodies to other lytic phase proteins, BMRF1 and BHRF1, have also been described in a few patients (25). Contrary to previous reports, we have obtained clear PCR evidence of EBV viral DNA in synovial tissues in 10 out of 11 patients (J. Edinger, unpublished data). Moreover, Koide et al. have recently established a RA synovium derived fibroblastoid cell line (with features of the synoviocyte type I), which expresses the latency genes EBNA1, EBNA2, and LMP1 and also expresses early antigen and viral capsid antigen in a small percentage of cells (26).

The envelopes of herpesviruses are complicated and presumably less rigid compared with those of VSV. The icosahedral core (17) is surrounded by a matrix and an envelope which derives from eukaryotic cell membranes. Future studies should address the ability of UV-treated EBV complexed with specific antibodies to elicit RF.

We thank Drs. P. Casali, M. Newkirk, and N. Chiorazzi for helpful discussions.

Address correspondence to David N. Posnett, The Immunology Program, Laboratory of Human Molecular Immunobiology and the Department of Medicine, Cornell University Medical College, New York, NY 10021.

Received for publication 26 March 1997.

References

1. Tighe, H., and D.A. Carson. 1997. Rheumatoid factors. *In* Textbook of Rheumatology. W.N. Lelley, E.D. Harris, S. Ruddy, and C.B. Sledge, editors. W.B. Saunders Company, Philadelphia. 241–249.
2. Casali, P., S.E. Burastero, M. Nakamura, G. Inghirami, and A.L. Notkins. 1987. Human lymphocytes making rheumatoid factor and antibody to ssDNA belong to Leu-1+ B-cell subset. *Science (Wash. DC).* 236:77–81.
3. Kunkel, H.G., V. Agnello, F.G. Joslin, R.J. Winchester, and J.D. Capra. 1973. Cross-idiotypic specificity among monoclonal IgM proteins with anti-γ globulin activity. *J. Exp. Med.* 137:331–342.
4. Slaughter, L., D.A. Carson, F.C. Jensen, T.L. Holbrook, and J.H. Vaughan. 1978. In vitro effects of Epstein-Barr virus on peripheral blood mononuclear cells from patients with rheumatoid arthritis and normal subjects. *J. Exp. Med.* 148:1429–1434.
5. Dresser, D.W., and A.M. Popham. 1976. Induction of an IgM anti-(bovine)-IgG response in mice by bacterial lipopolysaccharide. *Nature (Lond.).* 264:552–554.
6. Izui, S., R.A. Eisenberg, and F.J. Dixon. 1979. IgM rheumatoid factors in mice injected with bacterial lipopolysaccharides. *J. Immunol.* 122:2096–2102.
7. Karras, J.G., Z. Wang, L. Huo, R.G. Howard, D.A. Frank, and T.L. Rothstein. 1997. Signal transducer and activator of transcription-3 (STAT-3) is constitutively activated in normal, self-renewing B-1 cells but only inducibly expressed in conventional B lymphocytes. *J. Exp. Med.* 185:1035–1042.
8. Schroeder, H.W., Jr., J.L. Hillson, and R.M. Perlmutter. 1987. Early restriction of the human antibody repertoire. *Science (Wash. DC).* 238:791–793.
9. Kipps, T.J., B.A. Robbins, and D.A. Carson. 1990. Uniform high frequency expression of autoantibody-associated crossreactive idiotypes in the primary B cell follicles of human fetal spleen. *J. Exp. Med.* 171:189–196.
10. Mannik, M. 1982. Pathophysiology of circulating immune complexes. *Arthritis Rheum.* 25:783–787.
11. Victor, K.D., I. Randen, K. Thompson, O. Forre, J.B. Natvig, S.M. Fu, and J.D. Capra. 1991. Rheumatoid factors isolated from patients with autoimmune disorders are derived from germline genes distinct from those encoding the Wa, Po, and Bla cross-reactive idiotypes. *J. Clin. Invest.* 87:1603–1613.
12. Robbins, D.L., J. Skilling, W.F. Benisek, and R. Wistar, Jr. 1986. Estimation of the relative avidity of 19S IgM rheumatoid factor secreted by rheumatoid synovial cells for human IgG subclasses. *Arthritis Rheum.* 29:722–729.
13. Nemazee, D.A. 1985. Immune complexes can trigger specific, T cell–dependent, autoanti-IgG antibody production in mice. *J. Exp. Med.* 161:242–256.
14. Coulie, P.G., and J. Van Snick. 1985. Rheumatoid factor (RF) production during anamnestic immune responses in the mouse. III. Activation of RF precursor cells is induced by their interaction with immune complexes and carrier-specific helper T cells. *J. Exp. Med.* 161:88–97.
15. Holmdahl, R., C. Nordling, K. Rubin, A. Tarkowski, and L. Klareskog. 1986. Generation of monoclonal rheumatoid factors after immunization with collagen II–anti-collagen II immune complexes. An anti-idiotypic antibody to anti-collagen II is also a rheumatoid factor. *Scand. J. Immunol.* 24:197–203.
16. Fehr, T., M.F. Bachmann, E. Bucher, U. Kalinke, F. Di Padova, A.B. Lang, H. Hengartner, and R.M. Zinkernagel. 1997. Role of repetitive antigen patterns for induction of antibodies against antibodies. *J. Exp. Med.* In press.
17. Harrison, S.C., J.J. Skehel, and D.C. Wiley. 1996. Virus structure. *In* Fields Virology. B.N. Fields, D.M. Knipe, and P.M. Howley, editors. Lippincott-Raven Publishers, Philadelphia. 59–99.
18. Bachmann, M.F., and R.M. Zinkernagel. 1996. The influence of virus structure on antibody responses and virus serotype formation. *Immunol. Today.* 17:553–558.
19. Williams, R.C., and H.G. Kunkel. 1962. Rheumatoid factor, complement and conglutinin aberrations in patients with subacute bacterial endocarditis. *J. Clin. Invest.* 41:666–675.
20. Alspaugh, M.A., and E.M. Tan. 1975. Antibodies to cellular antigens in Sjogren's syndrome. *J. Clin. Invest.* 55:1067–1073.
21. Henle, G., E.T. Lennette, M.A. Alspaugh, and W. Henle. 1979. Rheumatoid factor as a cause of positive reactions in tests for Epstein-Barr virus–specific IgM antibodies. *Clin. Exp. Immunol.* 36:415–422.
22. Linde, A., J. Andersson, G. Lundgren, and B. Wahren. 1987. Subclass reactivity to Epstein-Barr virus capsid antigen in primary and reactivated EBV infections. *J. Med. Virol.* 21:109–121.
23. David-Ameline, J., A. Lim, F. Davodeau, M.A. Peyrat, J.M. Berthelot, G. Semana, C. Pannetier, J. Gaschet, H. Yie, J. Even et al. 1996. Selection of T cells reactive against autologous B lymphoblastoid cells during chronic rheumatoid arthritis. *J. Immunol.* 157:4697–4706.
24. Scotet, E., J. David-Ameline, M.-A. Peyrat, A. Moreau-Aubry, D. Pinczon, A. Lim, J. Even, G. Semana, J.-M. Berthelot, R. Reathnach et al. 1996. T cell response to Epstein-Barr virus transactivators in chronic rheumatoid arthritis. *J. Exp. Med.* 184:1791–1800.
25. Newkirk, M.M., J.B. Shiroky, N. Johnson, D. Danoff, D.A. Isenberg, C. Shustik, and G.R. Pearson. 1996. Rheumatic disease patients, prone to Sjogren's syndrome and/or lymphoma, mount an antibody response to BHRF1, the Epstein-Barr viral homologue of BCL-2. *Br. J. Rheumatol.* 35:1075–1081.
26. Koide, J., K. Takada, M. Sugiura, H. Sekine, T. Ito, K. Saito, S. Mori, T. Takeuchi, S. Uchida, and T. Abe. 1997. Spontaneous establishment of an Epstein-Barr virus infected fibroblast line from the synovial tissue of a rheumatoid arthritis patient. *J. Virol.* 71:2478–2481.