Periodicity During Recovery of Immune Response After Cyclophosphamide Treatment

By Amira Many and Robert S. Schwartz

The recovery of the immune response after a single dose of cyclophosphamide was studied in mice challenged with SRBC. Although the number of splenic antibody-forming cells was normal within 1 wk after the drug was given, it dropped below normal on two subsequent occasions (10 and 24 days after treatment). Disturbance of a feedback loop controlling the production of thymus-dependent lymphocytes was postulated.

PERIODIC VARIATIONS in the numbers of blood cells, as occur spontaneously in cyclic neutropenia, can be produced experimentally by bleeding,[1] the administration of hemolytic antibody,[2] irradiation,[3] or chemotherapy.[4] In this paper we will demonstrate oscillations in the number of antibody-forming cells following treatment of mice with cyclophosphamide.

MATERIALS AND METHODS

Eight-to-ten-wk-old C57Bl/6J mice were divided into 21 groups, each containing 6–20 animals. One day "zero" each experimental mouse received cyclophosphamide (Cytoxan, Mead Johnson), 150 mg/kg intraperitoneally. Sheep red blood cells (SRBC; 5×10^8) were administered intraperitoneally at different times before or after the cyclophosphamide to individual groups of mice. Ninety-six hr after the antigenic challenge each group of mice was killed and the antibody response was measured by the number of direct plaque-forming cells (PFC) in the spleen.[5] These cells are known to be producing hemolytic IgM antibodies, and are revealed when plated on an agar medium containing the antigen (SRBC) and complement.[5] Fifty C57Bl/6J mice were not treated with cyclophosphamide, and the number of PFC in their spleens 96 hr after challenge with SRBC provided the control value.

RESULTS AND DISCUSSION

Figure 1 shows the results. As reported previously,[6] the maximum degree of immunosuppression occurred when SRBC were administered either simultaneously with cyclophosphamide, or 24–48 hr previously. Although responsiveness to SRBC returned to normal about 1 wk after the single dose of cyclophosphamide, the number of splenic PFC spontaneously fell below normal on two subsequent occasions, giving rise to the oscillating curve shown in Fig. 1. The nadirs occurring on days 10 and 24 after cyclophosphamide treat-

Amira Many, M.D.: *Department of Hematology, Tel Hashomer Hospital, Ramat-Gan, Israel; formerly with the Department of Medicine, Tufts University School of Medicine, Boston, Mass.* Robert S. Schwartz, M.D.: *Clinical Immunology Service, New England Medical Center, Boston, Mass.*
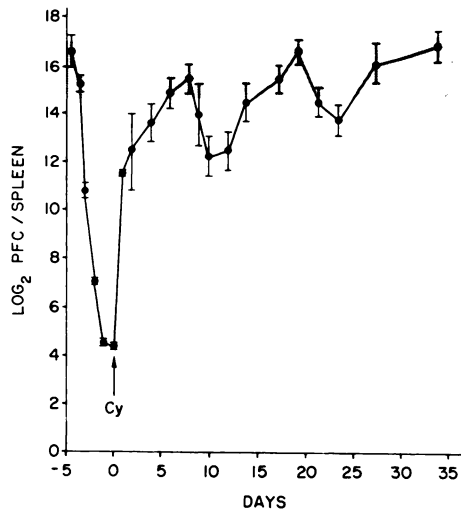
Fig. 1.—Recovery of PFC following a single injection of cyclophosphamide (Cy). Each point is the mean ± 2 SE of the splenic PFC found 96 hr after administration of 5×10^8 SRBC. Hatched area: mean ± 2 SE of splenic PFC found in control mice 96 hr after challenge with 5×10^8 SRBC.

ment were reproducible on three different occasions, during which different lots of cyclophosphamide and SRBC were used in vivo and different samples of guinea pig complement and agar were employed for the in vitro assay. Seasonal differences seem an unlikely explanation for the result, as it was reproduced at various times during the year.

Interpretation of the data must take into account that the immune response of the mouse to SRBC requires a cooperative interaction between at least two different cells. One of these is a thymus-dependent lymphocyte, the other a thymus-independent lymphocyte.[7] Studies of the immune response in vivo and in vitro have also implicated a third cell, the macrophage.[8] How these cells interact during the immune response is unclear, but the following are known: (1) The interaction of the two kinds of lymphocytes is synergistic rather than additive.[7] (2) The thymus-independent lymphocyte is the immediate precursor of the PFC; it is the cell that elaborates the antibody.[9] (3) The thymus-dependent lymphocyte does not differentiate into a PFC; instead, it is thought to possess an antigen-specific receptor—presumably an immunoglobulin on its membrane—that permits it to "display" immunogenic configurations to the thymus-independent lymphocyte.[10] The macrophage may, by enzymatic digestion, reduce the SRBC to small immunogenic units.

It seems unlikely that the macrophage can be the cause of the oscillations in PFC following treatment with cyclophosphamide since the immunologic function of this cell is not influenced by that agent.[11] Similarly, fluctuations in the thymus-independent lymphocyte are probably not responsible for the oscillations because it is also unaffected by cyclophosphamide during the immune response of mice to SRBC.[11,12] By contrast, both direct and indirect evidence strongly implicate the thymus-dependent lymphocyte as the target cell of cyclophosphamide in this system.[11,12] We therefore propose that the oscillating recovery of PFC following cyclophosphamide injury depends upon some special property of the population of thymus-dependent lymphocytes.

The production of this cell may be linked to a hormone, thymosin,[13] but

there is no convincing evidence that destruction of lymphocytes is associated with an increased production of thymosin. Adrenocorticosteroids may also participate in the control of lymphopoiesis in the mouse,[14] and the possibility is not excluded that these lymphocytolytic hormones are released consequent to the stress of cyclophosphamide treatment. Metcalf[15] has proposed that lymphocyte breakdown products within the thymus can regulate lymphopoiesis: the greater the cell destruction, the greater the stimulation of lymphopoiesis. Following treatment with cyclophosphamide, massive destruction of thymus lymphocytes occurs. Thus, perturbations in lymphocyte production could occur following cyclophosphamide treatment by an effect on thymosin, the release of corticosteroids, or the formation of large amounts of lymphocyte breakdown products.

Oscillations in antibody forming cells have been observed under other conditions. Following immunization of mice with SRBC or endotoxin, the number of splenic PFC rises and falls periodically.[16-18] This has been attributed to either the participation of two types of PFC in the immune response, the movement of cells out of the spleen, or feedback inhibition by antibody. By contrast with these experiments, ours determined the mode of recovery of PFC from inhibition by cyclophosphamide; all mice were tested for PFC at the same time during the immune response (96 hr after antigenic challenge). The effect we observed cannot be explained on the basis of feedback regulation by antibody because of the small amount of low-avidity antibody produced in the first 96 hr of the immune response; nor, as we mentioned, it is likely that macrophages or thymus-independent lymphocytes are involved. Instead, disruption of a feedback loop controlling the production of thymus-dependent lymphocytes seems the most reasonable explanation.

REFERENCES

1. Morley, A., and Stohlman, F., Jr.: Erythropoiesis in the dog: the periodic nature of the steady state. Science 165:1025, 1969.

2. Orr, J. S., Kirk, J., Gray, J. G., and Anderson, J. R.: A study of the interdependence of red cell and bone marrow stem cell populations. Brit. J. Haemat. 15: 23, 1968.

3. Morley, A., and Stohlman, F., Jr.: Periodicity during recovery of erythropoiesis following irradiation. Blood 34:96, 1969.

4. —, and —: Cyclophosphamide-induced cyclical neutropenia. New Eng. J. Med. 282:643, 1970.

5. Jerne, N. K., and Nordin, A. A.: Plaque formation in agar by single antibody-producing cells. Science 140:405, 1963.

6. Many, A., and Schwartz, R. S.: On the mechanism of immunological tolerance in cyclophosphamide-treated mice. Clin. Exp. Immun. 6:87, 1970.

7. Claman, H. N., Chaperon, E. A., and Triplett, R. F.: Thymus–marrow cell combinations—synergism in antibody production. Proc. Soc. Exp. Biol. Med. 122:1167, 1967.

8. Schwartz, R. S., Ryder, R. J. W., and Gottlieb, A. A.: Macrophages and antibody synthesis. Progr. Allerg. 14:81, 1970.

9. Nossal, G. J. V., Cunningham, A., Mitchell, G. F., and Miller, J. F. A. P.: Cell to cell interaction in the immune response. III. Chromosomal marker analysis of single antibody-forming cells in reconstituted, irradiated, or thymectomized mice. J. Exp. Med. 128:839, 1968.

10. Mitchison, N. A.: Antigen recognition responsible for the induction in vitro of the secondary response. Symp. Quant. Biol. 32:431, 1967.

11. Many, A., and Schwartz, R. S.: Drug-induced immunologic tolerance: site of action of cyclophosphamide. Proc. Soc. Exp. Biol. Med. 133:754, 1970.

12. Miller, J. F. A. P., Mitchell, G. F.: Cell to cell interaction in the immune response. V. Target cells for tolerance induction. J. Exp. Med. 131:675, 1970.

13. Goldstein, A. L., Slater, F. D., and White, A.: Preparation, assay and partial purification of a thymic lymphocytopoietic factor. Proc. Nat. Acad. Sci. U.S.A. 56: 1010, 1966.

14. Metcalf, D.: Adrenal cortical function in high- and low-leukemia strains of mice. Cancer Res. 20:1347, 1960.

15. —: The Thymus. New York, Springer-Verlag, 1966, p. 39.

16. Jehn, U., and Tannenberg, W.: Characterization of two different types of direct plaque forming cells. Clin. Exp. Immun. 6: 913, 1970.

17. Radovich, J., Hemingsen, H., and Talmage, D. W.: The immunologic memory of LAF_1 mice following a single injection of sheep red cells. J. Immun. 102:288, 1969.

18. Britton, S., and Möller, G.: Regulation of antibody synthesis against Escherichia coli endotoxin. I. Suppressive effect of endogenously produced and passively transferred antibodies. J. Immun. 100:1326, 1968.