PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 150, 664–668 (1975)

Hematologic Changes in Chronic Arthritis of Mice Induced by *Mycoplasma arthritidis*[1] (39101)

C. Q. EDWARDS, A. DEISS, B. C. COLE, AND J. R. WARD
(Introduced by G. E. Cartwright)

Department of Medicine, University of Utah College of Medicine, Salt Lake City, Utah 84132, and Salt Lake City Veterans Administration Hospital, Salt Lake City, Utah 84113

The anemia of chronic disorders occurs commonly in association with chronic infections, rheumatoid arthritis and cancer (1). The pathogenesis of this anemia involves abnormalities in the mechanisms which regulate red cell production, red cell survival time, and iron metabolism. Because these regulatory mechanisms are poorly understood, an experimental model would be highly useful in their study. Several methods have been used to produce inflammation in animals. Injection of bacterial endotoxin in rats produces abnormalities of iron metabolism which duplicate those in the anemia of chronic disorders, but the effect lasts only a day, and tolerance is produced by repeated daily injections (2). Sterile turpentine abscesses in dogs (3) and rats (4) produce somewhat more lasting effects but repeated turpentine injections are necessary, creating an intermittent rather than a chronic disease. Arthritis induced in rats by intradermal injection of adjuvant resolves gradually over a 60-day period; during this time, this represents a reasonably satisfactory model of the anemia of chronic disorders in man (5).

Cole *et al.* have reported that the intravenous (iv) injection of *Mycoplasma arthritidis* in mice produces a chronic polyarthritis (6). The histologic features of this arthritis are: (a) an initial acute phase with infiltration of the articular and periarticular tissues with polymorphonuclear leukocytes and mild hyperplasia of the synovium; (b) a mixed acute–chronic phase in which new areas of acute inflammation develop alongside lesions which resemble those seen in the chronic phase; (c) after 21 days, a chronic phase which is characterized by marked synovial proliferation, mononuclear cell infiltration, and pannus formation similar to the histologic features seen in human rheumatoid arthritis (7). The arthritis persists for at least 269 days. The present study was undertaken to examine the hematologic changes in these animals with chronic mycoplasmaf arthritis and to determine the suitability of this model for the study of the anemia ol chronic disorders. Unexpectedly, the anemia produced in these animals differed from the anemia of chronic disorders in man in that the concentration of plasma transferrin was increased rather than decreased.

Methods. Ten-week-old virgin female Swiss–Webster mice were fed Purina Laboratory Chow and water *ad libitum*. Suspensions of *M. arthritidis* strain $158P_{10}P_9$ were prepared and enumerated as previously described (8). Mice were injected iv with 10^9 colony-forming units (CFU) of the organism. The extent and severity of the arthritis was scored by the method of Cole *et al.* (7). Mice injected with 0.85% (w/v) sodium chloride served as controls.

On a random basis, groups of five mice from both the experimental and control groups were sacrificed at intervals through 109 days by light ether anesthesia and exsanguination by cardiac puncture. Hematologic measurements were made by standared methods (9) using heparinized blood. Red cell and white cell concentrations were determined using the Coulter particle counter model F_N. Plasma iron and total iron-binding capacity concentrations were measured by a published method (10) but using a 20-fold reduction in all reagent and sample volumes. Tissue nonheme iron was determined as the difference between total tissue

[1] Supported by the Veterans Administration, a graduate training grant (AM-5098), and research grants (AM-04489 and AM-02255) from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland.

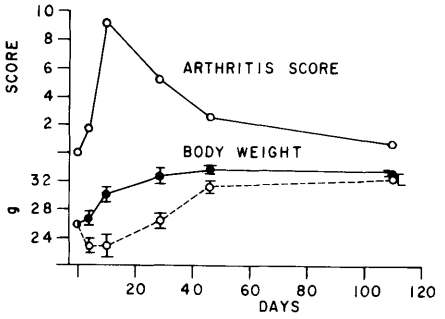

FIG. 1. Clinical course of arthritis and body wt in *M. arthritidis*-injected (○) and control mice (●). The points and bars refer to means ±1 SEM for groups of five mice.

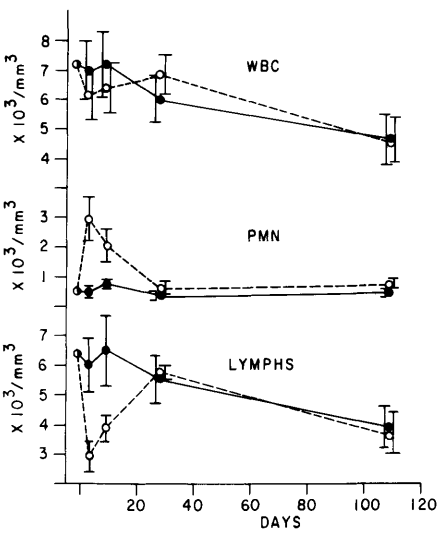

FIG. 2. Observations on absolute leukocyte concentrations in *M. arthritidis*-injected (○) and control mice (●). The points and bars refer to means ±1 SEM for groups of five mice. WBC refers to total leukocytes, PMN to polymorphonuclear neutrophils, and lymphs to lymphocytes.

iron (11) and heme iron measured as cyanmethemoglobin (12). Tissue sections were stained for ferric iron (13).

Results. Clinical course of arthritis. Arthritis was apparent as grossly visible swelling of joints at 4 days following injection and reached a mean score of nine by 10 days (Fig. 1). The arthritis slowly subsided over the ensuing 100 days. Initially, animals in this group failed to gain weight, but after Day 10 weight gain resumed and at 109 days the weights of the mice in the two groups were no longer significantly different (Fig. 1).

Variations in blood leukocytes. The WBC concentration was the same in the experimental animals as in the controls throughout the period of observation (Fig. 2). In the experimental animals, an increase in neutrophils was observed at Day 4. Metamyelocytes were present in the blood of experimental mice, reaching a maximum of 186 ± 74/mm³ (mean ± SE) on Day 10. Concomitantly with the neutrophilia, lymphopenia occurred (Fig. 2). The concentration of these cell types returned to control values by Day 29.

Variations in blood erythrocytes. Anemia developed in the animals with arthritis, the volume of packed red cells (VPRC) reaching a nadir of 41.5 ml/100 ml at 10 days following injection (Fig. 3). Because of wide variations within this group, this value is not significantly different from the control; however, significant differences between the mean VPRC of the two groups were found at Day 4 ($P < 0.01$) and Day 29 ($P < 0.02$). The VPRC gradually increased so that control levels were reached at Day 109. Initially the anemia was slightly microcytic, but by Day 29, the mean corpuscular volume (MCV) of the experimental and the control animals was the same (Fig. 3). The mean corpuscular hemoglobin concentration was the same in the two groups throughout the period of observation. Initially, reticulocytes were the same in the two groups, but at Day 29 the reticulocyte count of the experimental group was greater than that of the controls ($P < 0.01$) (Fig. 3). The time of the slight increase in reticulocytes and MCV (Day 29) coincided with the day on which the greatest increment in VPRC of the experimental group was observed.

Variations in iron metabolism. Coincident with the fall in VPRC, the plasma iron concentration (PI) decreased, and the total iron-binding concentration (TIBC) increased. The differences between the PI of the control and experimental groups were significant on Days 4 and 10 ($P < 0.005$ and < 0.05) and between the TIBC of the two groups on Days 10 and 29 ($P < 0.001$ and < 0.005). Both PI and TIBC returned to control levels at Day 46 (Fig. 4). These observations suggested iron deficiency, but

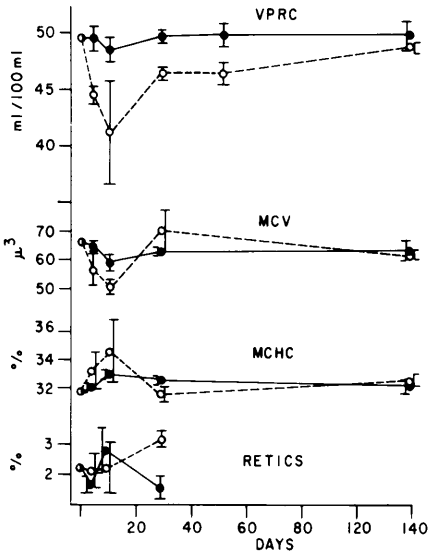

FIG. 3. Observations on erythrocytes in *M. arthritidis*-injected (○) and control mice (●). The points and bars refer to mean ±1 SEM for groups of five mice. VPRC refers to volume of packed red cells; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; Retics, reticulocytes.

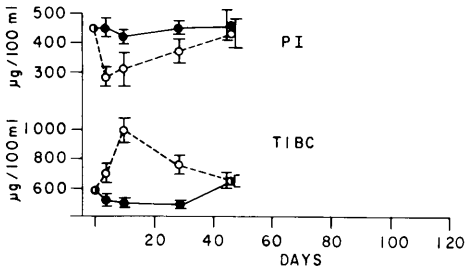

FIG. 4. Observations on plasma iron (PI) and total iron-binding capacity (TIBC) in *M. arthritidis*-injected (○) and control mice (●). The points and bars refer to mean ±1 SEM for groups of five mice.

there was no apparent site of external blood loss and stool guaiacs were negative. Stainable iron was plentiful in spleen sections of animals from both groups (Fig. 5). The mean nonheme iron content of spleens of additional control and experimental groups of four mice each was 220 ± 22.8 and 217 ± 8.5 μg/spleen, respectively, at Day 28; the mean VPRC, PI, and TIBC of this control group were 49.9 ml/100 ml, 461 μg/100 ml, and 505 μg/100 ml, respectively, and for this experimental group, 47.0 ml/100 ml, 338 μg/100 ml, and 783 μg/100 ml.

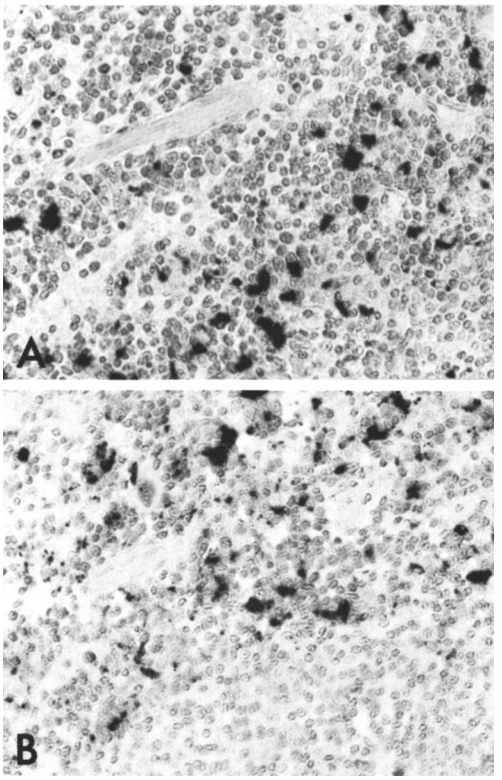

FIG. 5. Prussian blue stain showing reticuloendothelial iron in spleen of control (A) and *M. arthritidis*-injected mice (B).

Discussion. The mouse arthritis caused by iv injection of *M. arthritidis* consists of an initial phase of acute inflammation followed by a more persistent arthritis with histologic features of chronic inflammatory reaction. The severity of the disease is dependent upon the number of organisms injected. In the present experiments, the arthritis was of mild to moderate degree. While the pathogenesis of this arthritis is not clear, the early phase seems infectious. Eckner *et al.* have reported that mice injected with extracts prepared from *M. arthritidis* exhibited T lymphocyte depletion and suppression of mitogen-induced T cell blastogenesis (14). *M. arthritidis* has also been reported to interfere with the cellular and humoral immune mechanisms of the host (15, 16). These observations argue against an immunological reaction in the acute phase of the arthritis. However, the chronic phase of arthritis may be related to immunological injury due to stored antigens (17, 18).

The neutrophilia and lymphopenia observed coincided with the time during which arthritis was developing, and they subsided as the arthritis decreased. These cellular responses are similar to those produced by corticosteroids (19) and may represent a response to stress-induced endogenous cortisol release. What role, if any, T lymphocyte depletion by *M. arthritidis* plays remains to be determined (14).

The anemia of chronic disorders is usually mild. Morphologically, the erythrocytes are most often normocytic and normochromic; occasionally they are normocytic and hypochromic; and rarely, they are microcytic and hypochromic (1). The anemia is associated with several manifestations of deranged iron metabolism, namely: low plasma iron concentration and total iron-binding capacity, increased reticuloendothelial (RE) iron stores, and decreased numbers of sideroblasts in the marrow. The anemia observed in the present study simulated the anemia of chronic disorders as it occurs in man in that it was mild, the plasma iron concentration was decreased, and storage iron was plentiful. It differed from the anemia of chronic disorders of man in that the erythrocytes were microcytic and normochromic, and the total iron-binding capacity of the plasma was increased rather than decreased.

The increase in plasma total iron-binding capacity in these animals was unexpected. Hypertransferrinemia occurs in man in very few circumstances. Physiologically, it is seen in pregnancy and during infancy, and pathologically it occurs only in iron deficiency (20). None of these conditions applied to the experimental animals in this study. It is conceivable that the observed increase in TIBC may be ascribable to an abnormality in sex hormone metabolism in these female animals, but sex differences in the TIBC have not been observed in the anemia of chronic disorders in humans. The anemia observed in these animals, therefore, does not duplicate any anemia described in man.

The pathogenesis of the hypotransferrinemia in the anemia of chronic disorders in man is unexplained, although extensive studies have not been reported. Transferrin synthesis was normal *in vivo* in patients (21) and *in vitro* in livers from rats with turpentine-induced inflammation (22). A slight increase in the rate of degradation was observed in only a few of the patients in whom it was measured (21). Extravascular distribution of transferrin was no greater in patients with the anemia of chronic disorders than in normals (21). All of these observations were made in patients who had reached the stable phase of the anemia. To understand the mechanism of the hypotransferrinemia, it apparently will be necessary to make appropriate studies during the development of the anemia when a nonsteady state must exist.

Appropriate kinetic measurements that could have explained the observed increase in plasma transferrin concentration were not made in the present study. The hypertransferrinemia of iron deficiency has been ascribed to increased synthesis (23). It is generally believed that plasma transferrin concentration is regulated by the quantity of iron stores rather than by plasma iron concentration. Thus plasma transferrin concentration in man varies reciprocally with RE iron content (20, 24). That the plasma transferrin concentration is modified by factors other than the size of iron stores, however, is indicated by the hyptertransferrinemia that occurs in pregnancy, even in Bantu with excessive RE iron (25). In the present study, RE iron was plentiful as judged histochemically, and splenic nonheme iron was the same in experimental and control mice. It would be of interest to know if the differences between the anemia described in this study and the anemia of chronic disorders in man are only due to species differences in transferrin metabolism or are peculiar to infection with *Mycoplasma*. Similar studies utilizing mice and other means of producing inflammation, such as turpentine, have not been done; they should answer this question.

It is of interest that a role in the resistance to infection has been ascribed to transferrin (26). Bacterial growth requires availability of an optimal concentration of ionic iron. Low ionic iron concentrations in serum have been shown to be bacteriostatic. Dissociation of iron from transferrin is a function of the relative saturation of transferrin with iron. Low ionic iron concentrations,

therefore, may result from hypoferremia or hypertransferrinemia or, most effectively, from both. In the mouse, the percent saturation of transferrin normally is twice as great as in man (27). It may be speculated that in this species, the development of both hypoferremia and hypertransferrinemia would represent an important advantage to the host in resisting bacterial infection.

Summary. The arthritis caused by iv injection of *M. arthritidis* in mice was found to be associated with neutrophilia and lymphopenia without a change in the total WBC concentration. A mild anemia developed which was characterized by hypoferremia and plentiful RE iron but with an increased plasma total iron-binding capacity. This anemia therefore differs from the anemia of chronic disorders and indeed from any anemia which occurs in man.

We wish to express our appreciation to Arthur C. Claerhout, Mrs. Alice Tustison, and Ben J. Santistevan for technical assistance. Heparin was generously supplied by the Upjohn Company, Kalamazoo, Michigan.

1. Cartwright, G. E., *Semin. Hemat.* **3,** 351 (1966).
2. Kampschmidt, R. F., and Upchurch, H. F., *Proc. Soc. Exp. Biol. Med.* **110,** 191 (1962).
3. Cartwright, G. E., and Wintrobe, M. M., *J. Clin. Invest.* **28,** 86 (1949).
4. Quastel, M. R., and Ross, J. F., *Blood* **28,** 738 (1966).
5. Lukens, J. N., Cartwright, G. E., and Wintrobe, M. M., *Proc. Soc. Exp. Biol. Med.* **126,** 346 (1967).
6. Cole, B. C., Ward, J. R., Jones, R. S., and Cahill, J. F., *Infect. Immunol.* **4,** 344 (1971).
7. Cole, B. C., Cahill, J. F., Wiley, B. B., and Ward, J. R., *J. Bacteriol.* **98,** 930 (1969).
8. Golightly-Rowland, L., Cole, B. C., Ward, J. R., and Wiley, B. B., *Infect. Immunol.* **1,** 538 (1970).
9. Cartwright, G. E., "Diagnostic Laboratory Hematology," 4th ed. Grune and Stratton, New York (1968).
10. International Committee for Standardization in Hematology, *Blood* **37,** 598 (1971).
11. Gubler, C. J., Brown, H., Markowitz, H., Cartwright, G. E., and Wintrobe, M. M., *Blood* **7,** 1075 (1952).
12. Anderson, D., and Shoemaker, W. C., *Clin. Chem.* **11,** 442 (1965).
13. Luna, L. G., (ed.), "Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology," 3rd ed., p. 184. McGraw-Hill, New York (1968).
14. Eckner, R. J., Han, J., and Kumar, V., *Fed. Proc.* **33,** 769 (1974).
15. Kaklamanis, E., and Pavlatos, M., *Immunol.* **22,** 695 (1972).
16. Bergquist, L. M., Lau, H. S., and Winter, C. E., Abstracts, Annual Meeting, American Society of Microbiology, p. 86 (1973).
17. Harwick, H. J., Kalmanson, G. M., Fox, M. A., and Guze, L. B., *Proc. Soc. Exp. Biol. Med.* **144,** 561 (1973).
18. Cahill, J. F., Cole, B. C., Wiley, B. B., and Ward, J. R., *Infect. Immunol.* **3,** 24 (1971).
19. Bishop, C. R., Athens, J. W., Boggs, D. R., Warner, H. R., Cartwright, G. E., and Wintrobe, M. M., *J. Clin. Invest.* **47,** 249 (1968).
20. Bothwell, T. H., and Finch, C. A., "Iron Metabolism." Little, Brown, Boston (1962).
21. Jarnum, S., and Lassen, N. A., *Scand. J. Clin. Lab. Invest.* **13,** 357 (1961).
22. O'Shea, M. J., Kershenobich, D., and Tavill, A. S., *Brit. J. Haemat.* **25,** 707 (1973).
23. Tavill, A. S., and Kershenobich, D., *in* "Protides of the Biological Fluids. Proc. of the XIX Colloquium, 1971, Bruges" (H. Peeters, ed.), p. 489. Pergamon, Oxford (1972).
24. Weinfeld, A., *Acta Med. Scand. Suppl.* **177,** 427 (1964).
25. Gerritsen, T., and Walker, A. R. P., *J. Clin. Invest.* **33,** 23 (1954).
26. Weinberg, E. D., *Science* **184,** 952 (1974).
27. Kochan, I., *J. Bacteriol.* **100,** 64 (1969).

Received July 21, 1975. P.S.E.B.M. 1975, Vol. 150.