Idiopathic autoimmune neutropenia: Report of a case

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There are few case reports in the current literature that document antineutrophilic antibodies. A case is reported of a 64-year-old woman with idiopathic autoimmune neutropenia with clinical manifestations of recurrent, minor skin, conjunctival, and mucous membrane infections who was successfully treated with cyclophosphamide. Steroids were not successful at lower doses and only moderately effective at higher doses in this case. Splenectomy has been reported as variably useful in certain cases, but it is better if surgery can be avoided. Cyclophosphamide is a reasonable drug to employ since it has been effective in obtaining remissions in autoimmune thrombocytopenic purpura and other antibody-mediated cytopenias.

Idiopathic autoimmune neutropenia, unlike autoimmune hemolytic anemia and autoimmune thrombocytopenia, may be a rare entity, as suggested by Cline,[1] on the basis of infrequent case reports documenting antineutrophilic autoantibodies. Those cases which have been documented share similar clinical and laboratory findings with variable responses to the modalities generally used in the treatment of autoimmune thrombocytopenia, namely corticosteroids and splenectomy.[1-7]

Experience with cytotoxic agents in immune neutropenia refractory to corticosteroids is limited. The successful treatment of one patient with cyclophosphamide is reported here.

Report of case

A 64-year-old Caucasian woman was first seen in February 1975. She complained of skin abscesses, a persistent rash in body folds, oral canker sores, and intermittently inflamed eyes since November 1973. In February 1975, a leukocyte count of 1500/cu. mm. with an absolute granulocyte count of 250 was recorded. Drug history included the use of phenylbutazone for arthritic complaints unassociated with clinical swelling or inflammation for several weeks in 1971. Other medications included the employment of chlorpropamide and phenformin for diabetes since 1965, and lente insulin since 1969. She denied having previous blood transfusions or pregnancies, which may have caused active immunization. The patient was the only child of parents who died of natural causes.

A bone marrow aspirate revealed a moderate increase in fat, while the overall cellularity was judged to be normal. Megakaryocytes were of average number and morphology. There was a moderate granulocytic hypoplasia with a decrease in the normal 3:1 myeloid-erythroid ratio to 1:1. No megaloblastic maturation was evident. Leukocyte maturation progressed as far as the band stage of development with no evidence of polymorphonuclear neutrophils (Fig. 1). A scattered increase in mature plasma cells, well below 10 percent, was evident. The same phenomena was observed for eosinophils. Antinuclear antibody titer was negative while the rheumatoid factor was mildly increased to 1:160. Quantitative immunoglobulins were all elevated with values of 2,400 mg./100 ml. for IgG (n = 770-1,130), 460 mg./100 ml. for IgA (n = 60-200), and 210 mg./100 ml. for IgM (n = 90-170). The Westergren sedimentation rate measured 70 mm./hour. The liver/spleen scan and abdominal survey were unremarkable for organ enlargement on March 24, 1975 (Figs. 2 and 3), and September 20, 1976 (Figs. 4 and 5). The spleen was never clinically palpable.

Hematologic data and the methods of treatment are summarized in Table 1 and Fig. 6. Both depict starting and stopping dates of the different therapies. Methosarb and prednisone were initiated on March 21, 1975. Although a modest response was seen in the granulocyte count, prednisone was reduced on April 15, 1975, because of difficulty in controlling the patient's blood sugar levels. Methosarb was continued at the same dose. Prednisone was finally discontinued after the patient developed a severe eye infection on June 5. Methosarb was switched to Halotestin on June 11, 1975, because after almost 3 months, no response occurred. On August 14, 1975, oral androgens were stopped and twice daily doses of oral prophylactic penicillin started because of minor staphylococcal infections of the eyes and skin. Beginning October 22, 1976, parenteral androgens were administered weekly through January 1977. Penicillin was continued prophylactically. A repeat bone marrow aspirate was performed on September 23, 1976, and was unchanged from the prior study. In February 1977, the patient developed painful inflammatory skin ulcers in

TABLE 1. HEMATOLOGIC VALUES AFTER ADMINISTRATION OF PRESCRIBED DRUG.

Drugs	Date of change	Leukocytes (per cu. mm.)	Absolute granulocytes	Monocytes/lymphocytes (percent)	Platelets (per cu. mm.)
Methosarb (50 mg. P.O. t.i.d.) Prednisone (40 mg. P.O. daily)	3/21/75	1,100	88	2/90	138,000
Methosarb (50 mg. P.O. t.i.d.) Prednisone (10 mg. P.O. daily)	4/15/75	1,800	540	10/52	—
Methosarb (50 mg. P.O. t.i.d.)	5/5/75	900	396	8/48	—
Halotestin (10 mg. P.O. t.i.d.)	6/11/75	1,400	196	12/72	270,000
Penicillin (200,000 units P.O. b.i.d.)	8/14/75	1,500	(—)	(—)	Adequate
Deca-Durabolin (100 mg. I.M./week × 3 weeks then Depo-Testosterone 150 mg. I.M./week) + Penicillin (200,000 units P.O. b.i.d.)	10/22/76 — 1/77	1,400	84	4/74	Adequate
Cytoxan (125 mg. P.O. daily) × 4 weeks)	4/18/77	900	90	20/70	Adequate
No therapy	5/18/77	1,300	346	4/68	Adequate
No therapy	7/13/77	1,500	660	2/52	Adequate
No therapy	8/1/77	2,200	1,150	2/46	230,000
No therapy	11/4/77	4,100	3,116	1/23	198,000
No therapy	12/10/80	3,200	2,208	1/23	202,000
No therapy	3/24/81	3,500	2,415	5/24	161,000
No therapy	4/29/81	3,800	2,280	2/32	196,000
No therapy	6/22/81	2,900	1,769	5/31	171,000
No therapy	9/2/81	2,800	1,344	8/41	220,000
No therapy	12/17/81	4,700	3,149	3/23	218,000
No therapy	4/21/82	3,200	2,624	2/15	200,000

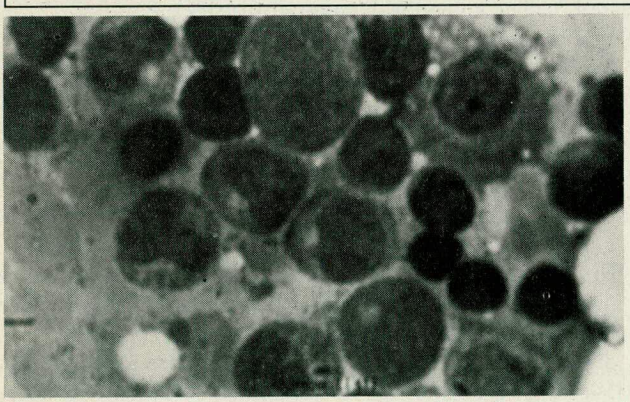
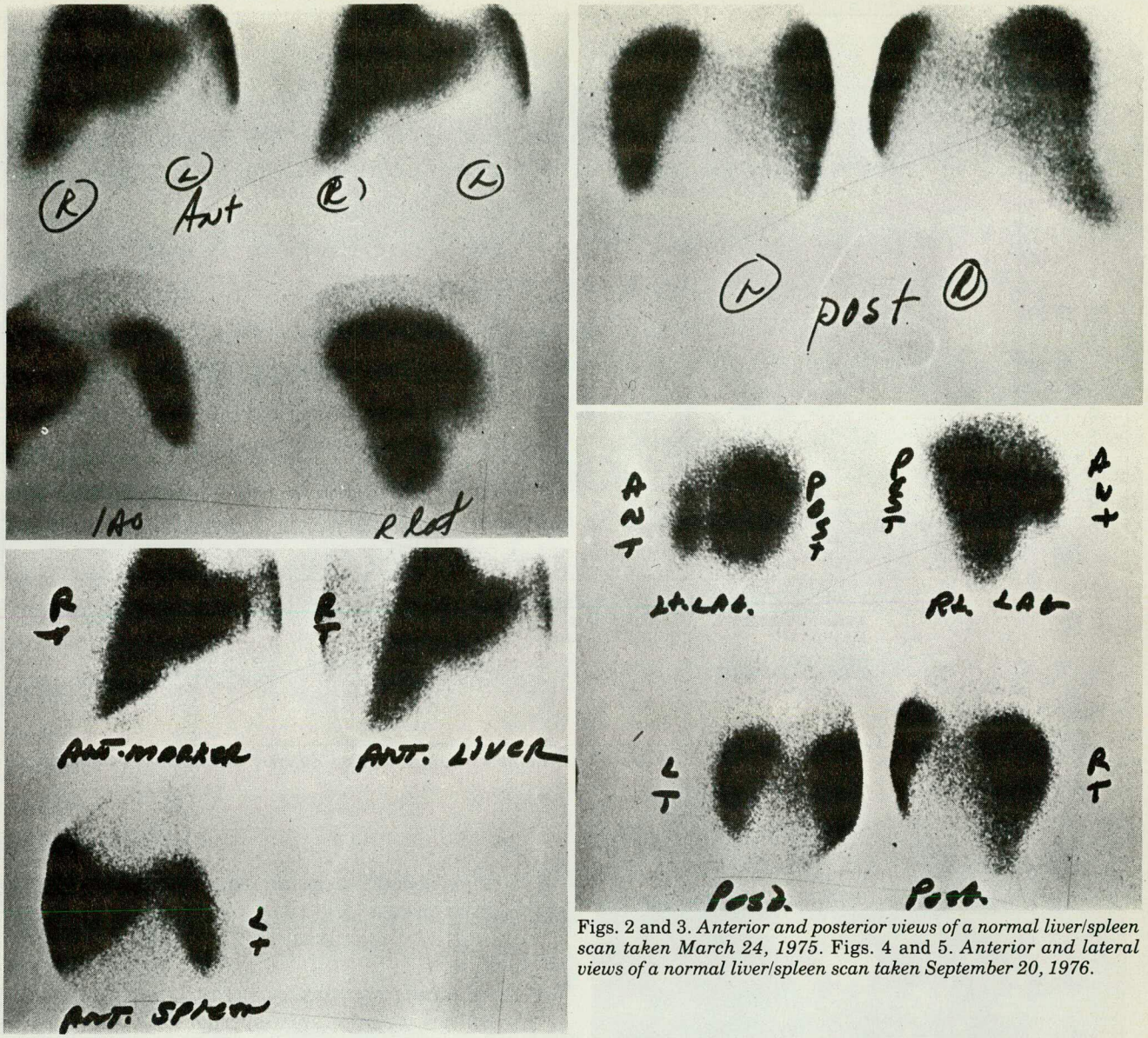


Fig. 1. *Pre-Cytoxan bone marrow aspirate. Note the absence of polymorphonuclear leukocytes.*

her right axilla and under her left breast. As suggested by Cline[1] in an earlier report, treatment with cyclophosphamide was initiated. On April 18, 1977, she began receiving oral, daily doses of 125 mg. This therapy was discontinued on May 18, 1977, because of nausea. One unit of plasma was pheresed and immediately frozen prior to the cytotoxic treatment. The patient was hospitalized in May 1977 because the skin ulcers worsened. Broad spectrum antibiotics and granulocyte transfusions were administered for 5 days and local ulcer care was given. Her absolute granulocyte count had already begun to rise by May 18, 1977, and it slowly continued to do so through her admission. By the time of discharge on July 13, 1977, it had reached 660/cu. mm. The patient had not received androgens for over 4 months. The bone marrow aspiration was repeated on November 4, 1977, when the granulocyte count reached 3,116 (Fig. 7). Normal cellularity was again evident, but the myeloid-erythroid ratio was now 3:1, with complete maturation and ratios of all granular elements including polymorphonuclear leukocytes.



Figs. 2 and 3. *Anterior and posterior views of a normal liver/spleen scan taken March 24, 1975.* Figs. 4 and 5. *Anterior and lateral views of a normal liver/spleen scan taken September 20, 1976.*

Plasma was again pheresed and frozen at this time for later evaluation. Serial studies for rheumatoid factor were subsequently negative. The last was taken on March 24, 1981. Follow-up examinations have failed to demonstrate palpable splenomegaly or evidence of small joint deformity. X-rays taken of her hands on July 5, 1978, were reviewed with the attending radiologist and rheumatologist. The films were non-diagnostic for rheumatoid changes (Figs. 8 and 9).

The patient was last seen on November 8, 1982. She offered no joint complaints, and her joints were clinically unchanged. She had not suffered any infections over the preceding 53 months. Her last complete blood count was performed on November 8, 1982. The leukocyte count was 3,500/cu. mm., with 61 percent polymorphonuclears; the hemoglobulin level was 13.6 gm./100 ml., and the platelet count was 221,000. Evaluation of the sedimentation rate, quantitative immunoglobulin, and rheumatoid factor were most recently repeated in June 1982. The sedimentation rate remains elevated at 40 mm./hour and the rheumatoid factor is still negative. Her IgG, IgA, and IgM are now 169 I.U./100 ml. (n = 79-167), 227 I.U./100 ml. (n = 49-220), and 109 I.U./100 ml. (n = 66-415), respectively.

Method

Peripheral blood neutrophils (designated test cells) were secured by a method described by Boxer and his colleagues.[2] "The cells were incubated with 133 μl of test serum per 10^7 neutrophils at 25° C for 30 minutes in the presence of 10 mM 2-deoxyglucose to inhibit the metabolism of the test cells; 15 ml of 0.15 M sodium chloride was then added to the tubes, which were suspended in 0.2 ml of modified Krebs-Ringer phosphate buffer, pH

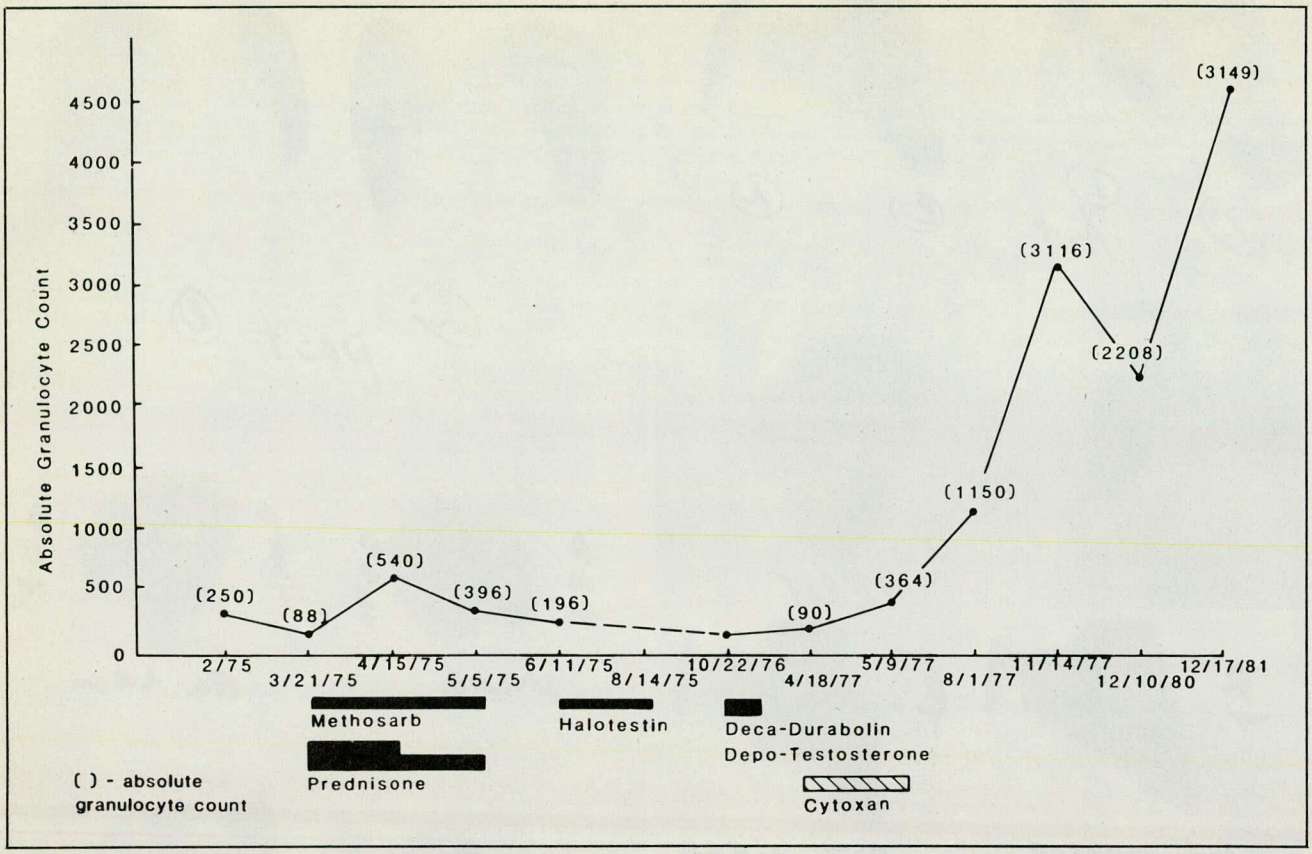


Fig. 6. *Graphic representation of the patient's absolute granulocyte count on the specific days different therapies were initiated and halted.*

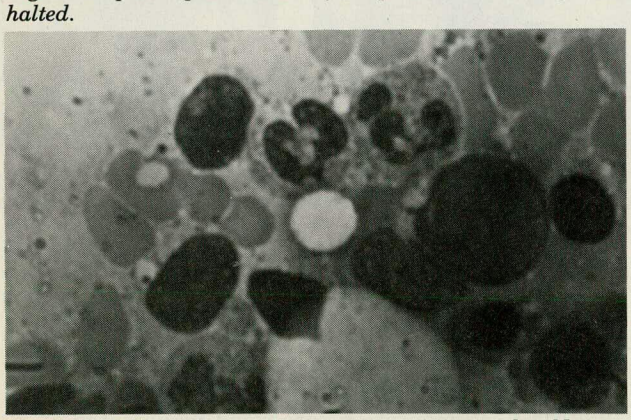


Fig. 7. *Post-chemotherapy bone marrow aspirate taken November 4, 1977. The reappearance of mature polymorphonuclears should be noted.*

7.4, at a final concentration of 8×10^6 cells per milliliter with 0.8 ml of 2×10^6 peripheral blood leukocytes in modified Krebs-Ringer phosphate buffer (indicator cells) containing a final concentration of 0.5 μCi of ^{14}C-1-glucose and 1 mM carrier glucose."[2] The initial rate of glucose oxidation by the phagocytic leukocyte was ascertained by scintillation spectrophotometry of evolved $^{14}CO_2$ after the test cells were incubated with the indicator cells for 30 minutes.[2] If the glucose oxidation rates exceeded two standard deviations above the control mean (n = 14) of 3,304 ± 1,474 cpm/10^7 cells/30 minutes, the test was considered positive.

The labeled staphylococcal protein A method[8] for the presence of antineutrophil antibodies was utilized to confirm the opsonic antineutrophil assay.

Results

Prior to the cyclophosphamide treatment, the serum of the patient contained opsonic antipolymorphonuclear neutrophil leukocyte activity that exceeded the control mean by 2.1 fold. After the therapy, the activity was only 1.1 fold greater than the control mean.

Confirmation that the antineutrophil activity found in the serum correlated with the presence of IgG-bound antineutrophil antibodies was obtained by identifying antineutrophil antibodies on the donor neutrophils with fluorescein-labeled staphylococcal protein A.[8]

Discussion

The case described represents one of several re-

ported in the literature on antibody-mediated neutropenia treated with cyclophosphamide. As far as I know, it is the second case that documents pre- and posttreatment antibody levels following a short course of cyclophosphamide with a long-term, unmaintained, clinical remission. Cline[1] presented the first case in 1976. My patient has had a 62-month, unmaintained remission of an immune-mediated neutropenia of unknown etiology. As of July 1979, when I communicated with Cline, his patient had been in an unmaintained remission for 36 months.

Leukocyte autoantibody primarily resides in the IgG fraction[8] and it seems to be most frequently associated with connective tissue disorders. Neutropenia may be seen in well over 50 percent of the patients with disseminated lupus erythematosus.[3] Felty's syndrome manifests leukopenia, splenomegaly, and chronic rheumatoid arthritis, and occurs in approximately 1 percent of rheumatoid patients.[9]

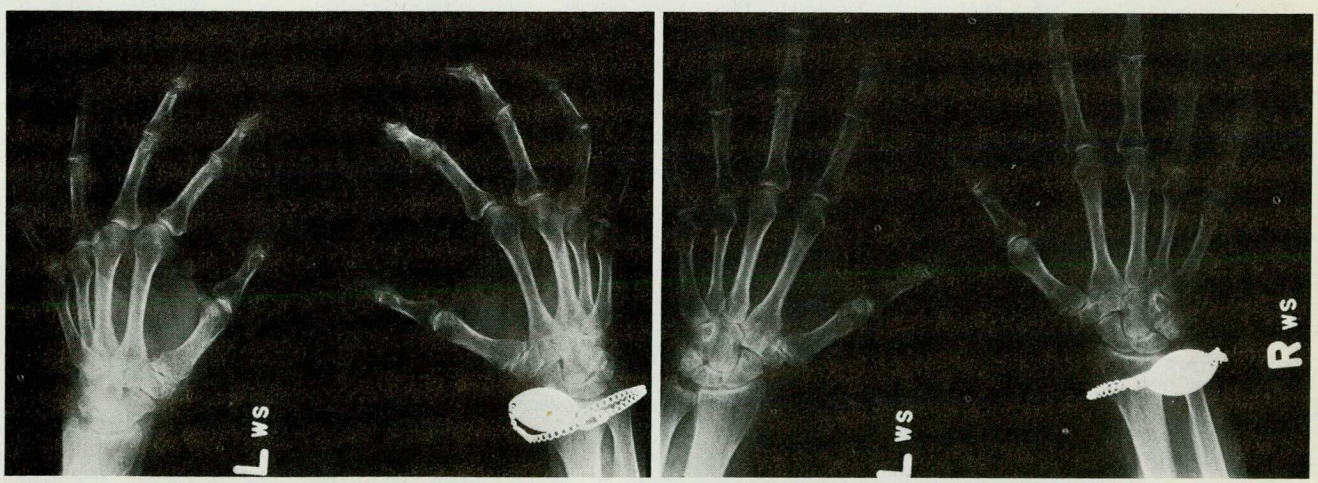
Hurd and coworkers[10] studied five patients with Felty's syndrome and repeated phagocytosis of circulating immune complex material by polymorphonuclear leukocytes, demonstrable as cytoplasmic inclusions under U-V microscopy after fluorescein-conjugation with goat anti-human IgG, IgM, IgA, and B_1C. One of the patients markedly improved after 5 months of treatment with cyclophosphamide. The patient did not display any evidence of vasculitis or leukopenia, and her cytoplasmic inclusions disappeared. Two additional cases of classic Felty's syndrome, successfully managed with cyclophosphamide, were discussed by Weisner and associates;[11] however, they did not provide demonstrable antibody data.

The etiology of immune neutropenia is often not known, but it may be related to drugs in certain cases.[12]

In an evaluation of 41 adults with chronic idiopathic neutropenia syndrome (CINS), Greenberg and his associates[13] found opsonizing antibodies in the serum of only three of the nineteen patients (16 percent) tested. This finding suggests that the enhanced peripheral removal of neutrophils related to the mechanism could have contributed to the neutropenia in only a minority of subjects. Dale and co-workers[14] performed in vivo leukokinetic studies indicating normal peripheral blood neutrophil survival and low incidence of opsonizing antibodies in patients with CINS. These patients tended to have chronic neutropenia despite lack of splenomegaly, histories of exposure to toxic drugs, evidence of systemic disease, or positive family histories of neutropenia.

The common denominators of the idiopathic case reports reviewed are comparatively mild infections, with predispositions toward the skin, conjunctiva, and mucous membranes.[1,2,4-7] Patients tend to manifest long histories of mild infections in these tissues. Bone marrow findings consistently reflect a marked decrease or absence of mature neutrophils as is the case with immune neutropenia associated with an obvious underlying disease. There appears to be an increase in the female-to-male ratio with all ages affected.

In vitro, bone marrow culture techniques have permitted assessment of granulocytic progenitor cells (CFU-GM) and the humoral stimulatory substance necessary for growth of these cells in vitro.[13] This substance has been termed colony-stimulating activity (CSA). Greenberg and his colleagues[15] noted that the cell cycle status of CFU-GM reflects the responsiveness for regeneration of myeloid precursor cells. Recent studies have shown the presence of cells capable of producing CSA within the microenvironment of the bone marrow (CSA_{BM})[16,17] in mouse and man.[17,18] And,



Figs. 8 and 9. *No evidence of rheumatoid changes can be found on these x-rays of the patient's hands taken July 5, 1978.*

according to Greenberg,[13] these local influences of granulocytopoiesis may be critical for the proliferation and maturation of granulocytic cells. His data[13] suggest that patients with a neutrophil count of less than 1,000/cu. mm. demonstrated significantly elevated proportions of CFU-GM in deoxyribonucleic acid synthesis in contrast to those neutropenic individuals having higher levels of neutrophils. However, significantly decreased values of CSA_{BM} were found in his patients and these amounts might have reflected suboptimal intramedullary levels of the stimulatory substance CSA in the chronic idiopathic neutropenia syndrome.

Albeit comparatively small as a demonstrable etiology for chronic idiopathic neutropenia, autoimmune disease has a growing body of evidence, both in vivo and in vitro, to make it a definite factor to consider. Complete or partial failure of production of hematopoietic cell lines may be caused by autoimmune mechanisms. Patients with aplastic anemia have responded to immunosuppressive therapy with antilymphocyte globulin or cyclophosphamide.[18]

Francis and coworkers[19] describe a patient with anemia and granulocytopenia who responded to immunosuppressive therapy, an apparent inhibitor of CSA_{BM} cells. The therapy does not directly act against CFU-GM. Immune neutropenia may be on a twofold basis: (1) an immune complex interacting with neutrophils as innocent bystanders triggering their aggregation and subsequent sequestration as cellular microemboli in the vascular bed of the kidneys and lungs;[20] and (2) mononuclear derived inhibitors of CSA_{BM} thus inhibiting CFU-GM.

As far as my patient is concerned, an immunologically mediated etiology has been demonstrated. The presence of antineutrophilic antibody in the pretreatment serum was illustrated by two independent methods serving as each other's control. The opsonic antineutrophil antibody method[2] and the fluorescein-labeled staphylococcal protein A method[8] were in agreement. The antineutrophilic antibody could not be demonstrated in the posttreatment serum.

We also believe that other known possible causes can be excluded because of several considerations. First, a drug etiology would appear unlikely since the patient was not taking any high-risk drugs for several years and a spontaneous recovery beginning shortly after the initiation of cyclophosphamide seems too farfetched. Secondly, oral and parenteral androgens had been stopped at least 4 months earlier, and androgens, with the help of erythropoietin, are known to directly stimulate hematopoiesis. It has no known effect on immunologically mediated cytopenias. Therefore, it is not surprising that while the patient was on androgens alone, her absolute granulocyte count remained unchanged. Thirdly, the leukocyte transfusions during her last hospitalization should not numerically affect the counts. Again, we showed that the granulocyte count had begun its initial rise on May 18, 1977, before this component therapy. The antibody data confirmed that the immune nature of the neutropenia and the proximity of response to the initiation of cyclophosphamide make it the logical causative factor for the improvement.

Felty's syndrome could not be confirmed despite the 1:160 rheumatoid titer on one occasion in the absence of joint deformities or overt splenomegaly. We believe that the same circulating immunoglobulin (IgG), and perhaps B_1C, attached to IgM, nonspecifically accounted for the positive rheumatoid factor as it attached to skin and neutrophils. The reason for the sudden flare-up of the painful skin lesions shortly following the initiation of the cyclophosphamide is only conjecture. Perhaps, the immunosuppressive agent blocked the reticuloendothelial uptake of immune complex material while also blocking further production. This material may have been deposited along the vascular intima of small vessels and perhaps, lysozyme was released from migrating neutrophils.

Finally, cyclophosphamide might be considered an alternative to steroid resistant Felty's syndrome prior to splenectomy. It should be considered a viable alternative since splenectomy fails to improve the degree of neutropenia in as many as 30 percent of all patients when they are reexamined 6 months or more after their respective operations.[21] More than one responsible mechanism is suggested by this figure. Perhaps, cyclophosphamide could generally suppress an inhibitor to CSA_{BM} as well as immune complex (IgG-neutrophil) hypersequestration. A controlled trial comparing steroid resistant cases in a two-armed fashion might be considered with one group undergoing splenectomy and the second group receiving cyclophosphamide. For example, splenectomy could be pitted against cyclophosphamide in a 4-week test.

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1. Cline, M.J., et al.: Autoimmune panleukopenia. N Engl J Med 295:1489-93, 30 Dec 76

2. Boxer, L.A., et al.: Autoimmune neutropenia. N Engl J Med 293:748-53, 9 Oct 75

3. Starkebaum, G., et al.: Autoimmune neutropenia in systemic lupus erythematosus. Arthritis Rheum 21:504-12, Jun 78

4. Thompson, D.S., et al.: Auto-immune neutropenia in an infant. Postgrad Med J 54:278-80, Apr 78

5. Nepo, A.G., et al.: Autoimmune neutropenia in an infant. J Pediatr 87:251-4, Aug 75

6. Lalezari, P., et al.: Chronic autoimmune neutropenia due to anti-NA2 antibody. N Engl J Med 293:744-7, 9 Oct 75

7. Valbonesi, M., et al.: Chronic autoimmune neutropenia due to anti-NA_1 antibody. Vox Sang 36:9-12, 1979

8. McCallister, J.A., Boxer, L.A., and Baehner, R.L.: The use and limitation of labeled staphylococcal protein A for study of antineutrophil antibodies. Blood 54:1330-7, Dec 79

9. Moore, R.A., et al.: Felty's syndrome. Long-term follow-up after splenectomy. Ann Intern Med 75:381-5, Sep 71

10. Hurd, E.R., LoSpalluto, J., and Ziff, M.: The role of immune complexes in production of the neutropenia of Felty's syndrome. J Rheumatol (Suppl) 1:105, 1974

11. Wiesner, K.B., et al.: Immunosuppressive therapy in Felty's syndrome [letter]. N Engl J Med 296:1172, May 77

12. Kanefsky, T.M., and Medoff, S.J.: Stevens-Johnson syndrome and neutropenia with chlorpropamide therapy. Arch Intern Med 140:1543, Nov 80

13. Greenberg, P.L., et al.: The chronic idiopathic neutropenia syndrome. Correlation of clinical features with in vitro parameters of granulocytopoiesis. Blood 55:915-21, Jun 80

14. Dale, D., et al.: Chronic neutropenia. Medicine 58:128-44, 1979

15. Greenberg, P.L., and Schrier, S.L.: Granulopoiesis in neutropenic disorders. Blood 41:753-69, Jun 73

16. Chan, S.H., and Metcalf, D.: Local production of colony-stimulating factor within the bone marrow. Role of nonhematopoietic cells. Blood 40:646-53, Nov 72

17. Greenberg, P.L., Mara, B., and Heller, P.: Marrow adherent cell colony-stimulating activity production in acute myeloid leukemia. Blood 51:362-78, Aug 78

18. Baran, D.T., Griner, P.F., and Klemperer, M.R.: Recovery from aplastic anemia after treatment with cyclophosphamide. N Engl J Med 295:1522-3, 30 Dec 76

19. Francis, G.E., et al.: Response of neutropenia and anaemia to immunosuppressive therapy. Report and bone marrow studies. Clin Lab Haematol, pp. 203-14, 1979

20. Caligaris-Cappio, F., Camussi, G., and Gavosto, F.: Idiopathic neutropenia with normocellular bone marrow. An immune-complex disease. Br J Haematol 43:595-605, 1979

21. Dancey, J.T., and Brubaker, L.H.: Neutrophil marrow profiles in patients with rheumatoid arthritis and neutropenia. Br J Haematol 43:607-17, Dec 79

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