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BRIEF REPORT

MONOCLONAL-ANTIBODY THERAPY IN SYSTEMIC VASCULITIS

PETER W. MATHIESON, M.R.C.P.,
STEPHEN P. COBBOLD, PH.D.,
GEOFFREY HALE, PH.D.,
MICHAEL R. CLARK, PH.D.,
DAVID B.G. OLIVEIRA, PH.D., M.R.C.P.,
C. MARTIN LOCKWOOD, F.R.C.P.,
AND HERMAN WALDMANN, PH.D., M.R.C.P.

MONOCLONAL antibodies are potentially useful therapeutic agents in a variety of immunologically mediated diseases, offering the theoretical advantage of selective attack on cells implicated in the immunopathogenesis of these disorders. Antibodies to surface markers on lymphocytes, particularly T cells, have already demonstrated efficacy both in animal models and in clinical allograft rejection.[1-3] For this purpose, monoclonal antibodies can be used either to block vital receptors for antigen, adhesion, or growth factors or to block target cells by harnessing the various natural effector systems (complement and accessory cells) that are activated by the Fc regions of cell-bound antibodies. The optimal use of these natural effector systems depends on the specificity and isotype of the therapeutic antibody.[1] Since the administration of xenogeneic antibodies to humans tends to result in the neutralization of the antibodies by an antiglobulin response, long-term or repeated use of such therapeutic antibodies can be possible only if the agents can be rendered poorly immunogenic.

We report the use of two monoclonal antibodies to establish a long-lasting remission in a patient with previously intractable systemic vasculitis. The first antibody, Campath-1H, is a genetically engineered "humanized" form of a rat antibody that belongs to the CDw52 cluster and is capable of depleting human lymphocytes.[4-7] The second is a rat antibody to the human CD4 molecule on T helper cells; the antibody can interfere with the function of this cell-surface adhesion receptor. In this preliminary study, we observed that the combination of these two antibodies improved disease status and preempted any immune response to the human or rat immunoglobulins. This form of combination therapy may be of value in the management of human autoimmune diseases.

From the Departments of Medicine (P.W.M., D.B.G.O., C.M.L.) and Pathology (S.P.C., G.H., M.R.C., H.W.), University of Cambridge, Addenbrooke's Hospital, Cambridge, United Kingdom. Address reprint requests to Dr. Mathieson at the Office of the Regius Professor of Physic, Clinical School, Addenbrooke's Hospital, Hills Rd., Cambridge CB2 2QQ, United Kingdom.

Dr. Mathieson is a Medical Research Council Training Fellow. Dr. Oliveira is a Lister Institute Research Fellow. Dr. Lockwood is a Wellcome Senior Lecturer.

METHODS

Monoclonal Antibodies

Campath-1H

The Campath-1 antigen is present on virtually all lymphoid cells and monocytes, but not on other cell types.[8,9] Various rat IgM and IgG monoclonal antibodies to this antigen have been produced.[10,11] Treatment with rat antibody, however, is likely to induce an antirat globulin response that would limit therapy. This has led to the development of Campath-1H, a humanized version of the rat monoclonal antibody that is produced by transplantation of the hypervariable regions of the rat antibody into normal human immunoglobulin genes.[6]

Rat CD4

This rat IgG2b monoclonal antibody was made by fusing the rat Y3-Ag1.2.3 myeloma cell line[12] with spleen cells from a PVG-RT1^u rat immunized with a rat T-cell line (NB2) that had been transfected with the human CD4 gene. A clone was selected (YNB46.1.8) that produced a rat IgG1 antibody able to recognize human CD4 on the transfectant and that was specifically blocked by certain known mouse anti–human CD4 antibodies (13B.8.2, VIT4, MT321, B264/123, and CLB-T4/1) but not by others (Leu-3a, OKT4, and 66.1), in a manner consistent with the known pattern of epitopes on the CD4 molecule.[13] The rat IgG2b–producing clone (YNB46.1.8SG2B1.19) was derived by selecting class-switch variants by the method of sibling selection.[11] Monoclonal antibody was obtained by growing the cell line in an Acucyst Jr (Endotronics, Minneapolis) and partially purified by 50 percent ammonium sulfate precipitation, followed by ion-exchange chromatography on Fast-Flow S (Pharmacia, Piscataway, N.J.).

Antiglobulin Assays

Responses to the Campath-1H and CD4 monoclonal antibodies were estimated with a capture enzyme-linked immunosorbent assay, as described elsewhere.[14]

In Vitro Tests of Lymphocyte Function

To monitor the immunosuppressive effects of the Campath-1H

treatment, simple nonspecific tests of T-cell and B-cell function were performed on peripheral-blood lymphocytes sequentially.

Reactivity to Concanavalin A

Peripheral-blood lymphocytes from the patient and a normal control were separated by density-gradient centrifugation. Proliferation assays were performed by incubating the cells in tissue-culture medium (Iscove's modification of Dulbecco's medium, containing 5×10^{-5} M 2-mercaptoethanol and 10 percent fetal-calf serum) in 96-well tissue-culture plates (Flow Laboratories, Rockville, Md.) at 2×10^{5} cells per well with various concentrations of concanavalin A (Sigma, St. Louis), a nonspecific T-cell mitogen. The cells were incubated for 96 hours at 37°C in an atmosphere containing 5 percent carbon dioxide, and proliferation was assessed according to the rate of incorporation of tritiated thymidine during the last 18 hours of incubation.

Production of Antibody to Tetanus Toxoid by B Cells

Antibody to tetanus toxoid was produced in B cells by a method adapted from that of Sedgwick and Holt.[15] Briefly, peripheral-blood lymphocytes from the patient and the normal control were incubated for five days with pokeweed mitogen (Sigma). The cells were then incubated in flat-bottomed 96-well plates that had been coated with tetanus toxoid (the test wells) or phosphate-buffered saline containing 1 percent bovine serum albumin (the controls). The wells were then washed and incubated with alkaline phosphatase–conjugated antihuman IgG (Sigma). After further washing, substrate was added (5-bromo-chloro-indolyl-phosphate [Sigma], immobilized in 0.6 percent agar). Specific B cells that produced antibody to tetanus toxoid could then be enumerated when the resultant blue spots were counted.

Case Report

The patient's illness has spanned 25 years, beginning in 1965 at the age of 40, and has been characterized by fever, rash, polyarthritis, myalgia, pericarditis, pleurisy, and episcleritis. Initially, the symptoms were controlled by prednisolone (5 to 20 mg daily). Dapsone and azathioprine each produced some improvement, but they were stopped because of adverse effects (hemolytic anemia and hepatic dysfunction, respectively). Cyclophosphamide (100 mg daily) caused leukopenia, and the patient was never able to tolerate more than 50 mg daily. In September 1987 he had multiple vertebral collapse, and a gibbus developed. In November 1987 the patient improved after five plasma exchanges; thereafter, he received monthly plasma exchanges that improved his skin lesions and systemic symptoms temporarily, but the exchanges were poorly tolerated.

Blood tests showed only an acute-phase response, with normal serum complement levels and no evidence of circulating immune complexes. In 1986 antithyroglobulin antibodies developed, and the patient was found to have hypothyroidism. A muscle biopsy in 1986 and skin biopsies in 1986 and 1988 showed an active vasculitis (Fig. 1A and 1B). Immunohistochemical analysis showed a predominance of CD8+ T lymphocytes (Fig. 1C). A test for antineutrophil cytoplasmic antibody[16] was positive on one occasion in September 1987, although the pattern of reactivity was atypical, as documented elsewhere.[17]

In November 1988 the patient had high, fluctuating fevers, anorexia, weight loss, arthralgia, and a bright red rash. He was given Campath-1H (2 mg daily) by intravenous injection for eight days. The rash improved dramatically within 48 hours (Fig. 2), and the fever resolved; however, this remission lasted only 10 days. Two further courses of Campath-1H induced similar short-lived remissions. In February 1989 Campath-1H (2 mg daily) was given for 3 consecutive days, followed by intravenous injection of the CD4 antibody (20 mg daily) for the next 12 days (total CD4-antibody dose, 240 mg). The sequential use of the two antibodies was followed by complete remission of the fever and other symptoms.

The monoclonal-antibody therapy was tolerated well. It was a month after the sequential antibody treatment before CD4-positive lymphocytes were detectable in the circulation, and the CD4/CD8 (helper/suppressor) ratio remained profoundly depressed. Despite this, the patient remains well 12 months after completing the sequential monoclonal-antibody treatment. In the intervening period, his only drug therapy has been an adrenal-replacement dose of corticosteroid; he has had occasional short-lived skin lesions similar

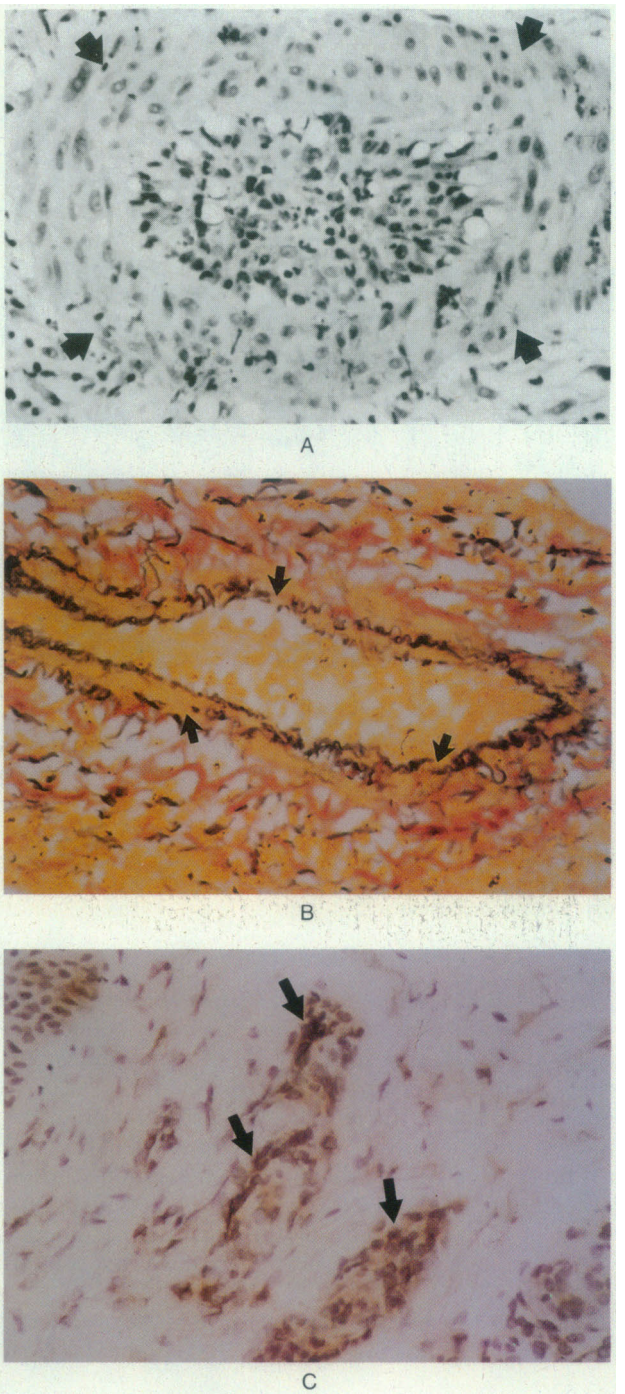

Figure 1. Skin-Biopsy Specimens Obtained from the Patient in November 1988.

Panel A shows the lumen of the dermal artery occluded by inflammatory cells. Arrows indicate the arterial wall (hematoxylin and eosin, ×90). Panel B shows the disruption of the internal elastic lamina of the dermal artery (arrows) (elastin, ×20). Panel C shows perivascular infiltration of CD8+ T lymphocytes (arrows) (immunoperoxidase technique with OKT8 antibody, ×20).

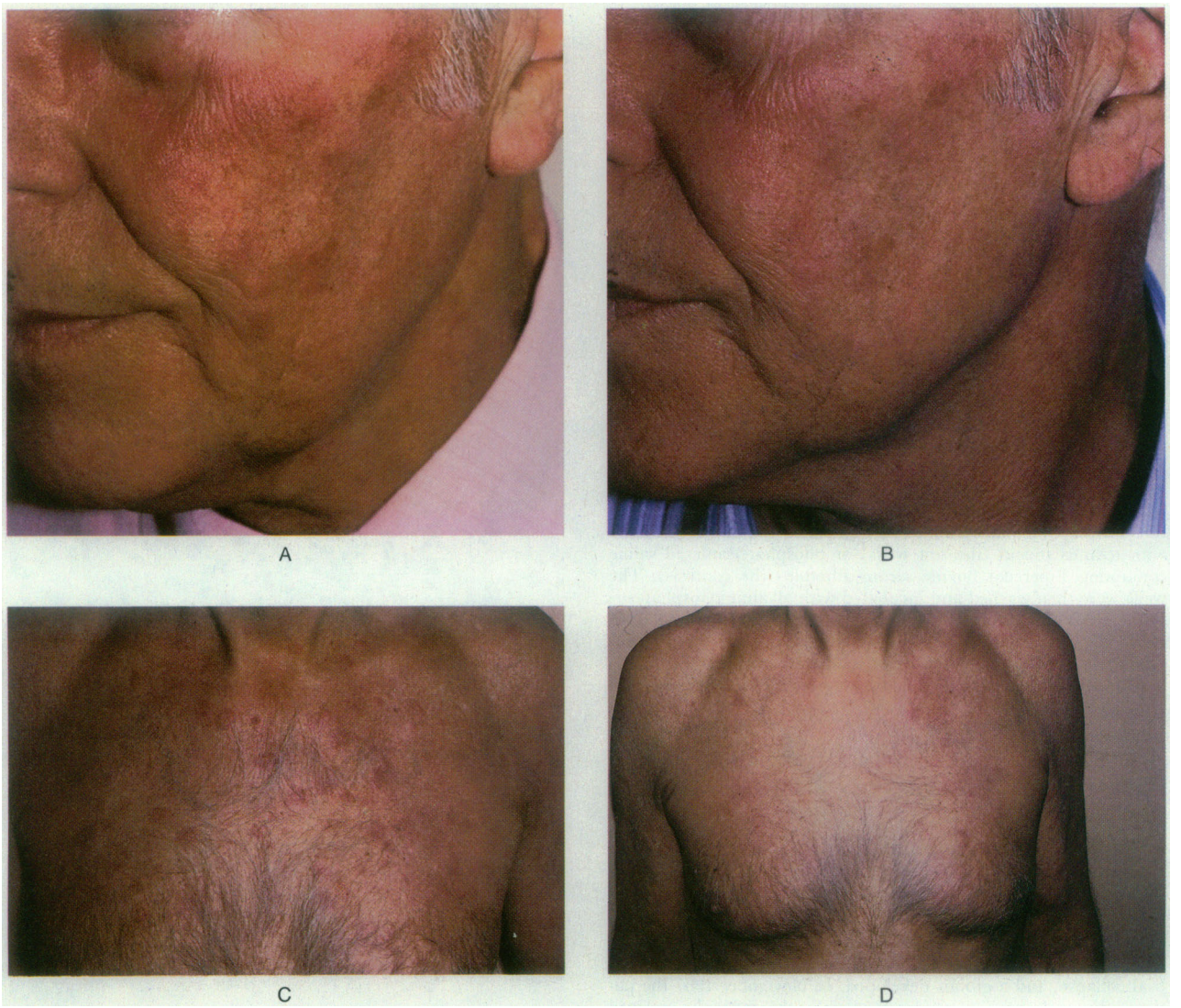

Figure 2. Effect of Campath-1H on the Patient's Rash.
The photographs in Panels A and C were taken immediately before treatment, and those in Panels B and D 48 hours after the first dose.

to those observed during the previous rash, but he has had no fever or constitutional upset and has returned to work. He has had no opportunistic infections.

Results

Antiglobulin assays showed that the patient had no detectable antiglobulin response to either monoclonal antibody — Campath-1H or CD4 — at any time up to three months after completing treatment.

Figure 3 shows the lymphocytic proliferation in the patient and the normal control in response to concanavalin A (1 μg per milliliter). After the administration of Campath-1H, there was a rapid and complete suppression of the response of the patient's peripheral-blood lymphocytes, even during a clinical relapse (on day 16).

Figure 4 shows the number of B cells producing antibody to tetanus toxoid per 10^6 cells plated for each subject. After the administration of Campath-1H, there was a long-lasting suppression of this B-cell response that continued after the patient relapsed.

Discussion

Since this patient had severe systemic vasculitis that was difficult to control with conventional therapy and had experienced numerous adverse effects of various immunosuppressive treatments, he seemed a reasonable candidate for monoclonal-antibody therapy. Rat-antibody therapy carries the risk of inducing an anti-rat globulin response that could lead to a serum sickness–like reaction after repeated courses and to the neutralization of the antibody, rendering it ineffective. Thus, the humanized monoclonal antibody Campath-1H was particularly attractive, since it seemed likely that long-term treatment would be required. This antibody has been used successfully to induce remis-

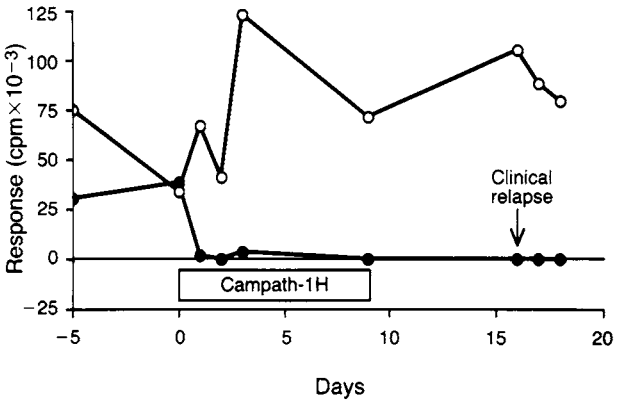

Figure 3. Response of Peripheral-Blood Lymphocytes to Incubation with Concanavalin A (1 μg per milliliter) in the Patient Receiving Campath-1H (Solid Circles) and a Control (Open Circles).
Response is shown as counts per minute (cpm) of incorporated tritiated thymidine. The patient's clinical relapse occurred on day 16.

sion in non-Hodgkin's lymphoma,[7] but it has not been used previously in systemic vasculitis. In our patient, it induced a short-lived remission. The humanization process does not preclude the development of an antiidiotypic response to the antibody, which in theory could neutralize the antibody after the first course of treatment, but this patient had neither an antiidiotypic nor an antiallotypic response[14] to Campath-1H. Repeated courses of this antibody did not diminish its ability to induce a remission, but on each occasion relapse followed rapidly. The results of tests of T-cell and B-cell function correlated poorly with the clinical status.

The CD4 monoclonal antibody is of rat origin and has not been used previously in humans. In experimental studies, antibodies to the CD4 molecule have shown beneficial effects in a number of autoimmune diseases.[18-24] A mouse monoclonal anti-CD4 antibody

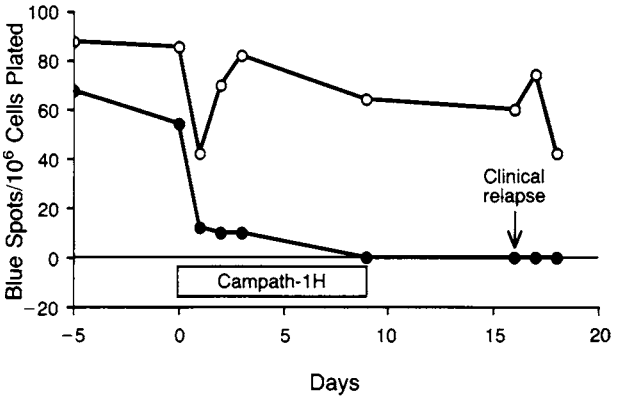

Figure 4. Number of B Cells Producing Antibody to Tetanus Toxoid in the Patient Receiving Campath-1H (Solid Circles) and a Control (Open Circles).
The number of cells was determined by counting the number of blue spots per 10^6 cells in the wells of the tissue-culture plates. The patient's clinical relapse occurred on day 16.

has been given to four patients with rheumatoid arthritis and one with psoriatic arthritis, with apparent short-term improvements in clinical status.[25] CD4 antibodies have induced lasting immunologic tolerance in experiments in animals.[1]

Our aim was first to deplete the bulk of lymphocytes with Campath-1H and then to apply the CD4 antibody to the remaining cells in the hope of inducing tolerance to the putative autoantigen. A similar approach has recently been effective in an experimental model of autoimmune disease, collagen-induced arthritis.[26] In our patient a useful remission was induced, lasting 12 months so far. The monoclonal-antibody therapy produced no important adverse effects, and there has been no evidence of an antirat globulin response. We cannot be certain whether we have merely ameliorated the effector arm of the autoimmune response or whether the monoclonal-antibody therapy might have had some more fundamental effect in restoring self-tolerance. In any event, the monoclonal antibodies have apparently altered the natural history of this patient's disease, which was previously difficult to manage because of an incomplete response to conventional therapy combined with an accumulation of adverse effects from numerous immunosuppressive regimens. Such "tolerance therapy," with a debulking agent such as Campath-1H followed by CD4 monoclonal antibodies, may be applicable to other autoimmune conditions as well.

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CASE RECORDS
OF THE
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Weekly Clinicopathological Exercises

FOUNDED BY RICHARD C. CABOT

CASE 30-1990

PRESENTATION OF CASE

A 47-year-old man was admitted to the hospital because of a rash and fever.

He was well until four days earlier, when a pruritic rash developed over the upper arms. The patient recalled exposure to poison ivy one week earlier and assumed that he was experiencing another bout of poison-ivy dermatitis. The rash spread to involve his legs, anterior chest, back, and abdomen, without conjunctival or oral involvement; a dry cough developed. By that time the rash consisted of papules, pustules, and plaques. Two days before admission the patient consulted a dermatologist; a Tzanck test was reported to be positive, and acyclovir was begun. On the same day fever developed, with a shaking chill and recurrent headaches. The patient was admitted to the hospital.

The patient was an electrician. He resided with his wife and three adult children, all of whom were well. An appendectomy was performed 20 years earlier. There was a 30–pack-year history of cigarette smoking, discontinued 10 years before entry; he consumed four beers daily. He used naproxen in recent weeks for polyarthralgia that dated back three years. Several weeks before admission he attended a birthday party with 12 children, 3 to 5 years old, none of whom were known to be ill at the time or thereafter. He recalled having had rubeola in childhood. He had traveled to Florida and New Orleans within the previous year. There was no history of photophobia, stiff neck, past varicella, chest pain, dyspnea, pallor, fatigue, easy bruising, epistaxis, risk factors for human immunodeficiency virus (HIV) infection, intravenous drug abuse, rheumatic fever, tuberculosis, or exposure to animals or arthropods.

The temperature was 39.1°C, the pulse was 92, and the respirations were 20. The blood pressure was 120/80 mm Hg.

On examination the patient appeared well. A widespread, symmetric rash was present on the arms, dorsal aspects of the hands, shoulders, upper chest, and thighs (Fig. 1); most of the lesions were edematous papules and purple–red plaques; their surfaces were irregularly studded with small papules, pustules, and crusts (Fig. 2); a few plaques on the hands were surmounted by solitary pus-filled blisters; in some areas small plaques coalesced to form larger ones (Fig. 3); other lesions included follicular pustules, nonfollicular pustules, and pale, edematous dermal papules that resembled vesicles but contained no fluid. The lesions spared the palms, soles, face, mucous membranes, and genitalia. No lymphadenopathy was found. The head and neck were normal; a few inspiratory crackles were heard at both lung bases. The heart was normal. Abdominal examination revealed that the edge of the liver descended 4 cm below the right costal margin; the spleen was not felt. The extremities and joints were normal. Neurologic examination was negative.

The urine was normal except that the sediment contained 15 white cells and a few bacteria per high-power field. The hematocrit was 36.1 percent; the white-cell count was 9900, with 59 percent neutrophils, 15 percent band forms, 15 percent lymphocytes, 9 per-