investigation sarcoid spleen suspension has induced an even greater contrast in patterns of leucocyte migration between a group of patients with Crohn's disease and a group with ulcerative colitis than between the former and a miscellaneous group posing no problem in differential diagnosis.

Only one out of 17 untreated patients with definite or probable Crohn's disease showed leucocyte migration within the range found for nine patients with ulcerative colitis. That the two diseases may react differently in migration inhibition systems has already been shown by Bendixen (1967), who found that inhibition of leucocyte migration in the presence of mucosal extracts occurred in ulcerative colitis but not in Crohn's disease, and by Dykes (1970), who, using a closely related technique, has shown that this pattern is reversed if faecal extracts or small-bowel contents are substituted as "antigen."

In order to gain acceptance as a reliable complementary test in the diagnosis of Crohn's disease the measurement of leucocyte migration in the presence of sarcoid spleen suspension must first be shown to give comparable results with different batches of suspension prepared from both K19 and other sarcoid spleens. The mechanism of migration inhibition in this system is the object of current investigation, and in this context it is of interest that the results of a concurrent study in which the same strict criteria of our earlier reports were used (Mitchell *et al.*, 1969, 1970) have thus far shown positive Kveim tests with a carefully validated test suspension (K12) in 8 out of 34 patients with a clinical diagnosis of ulcerative colitis and in 5 out of 12 with coeliac disease, and that closely similar results have been obtained following simultaneous Kveim tests among the same patients with another widely used (Hurley) test suspension (Mitchell, Cannon, Dyer, Hinson, and Willoughby, to be published). It is, therefore, already necessary to consider the possibility that the cutaneous and in-vitro responses to sarcoid spleen suspensions may be partially or wholly distinct.

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PRELIMINARY COMMUNICATIONS

Mechanisms for Eosinophilic and Neutrophilic Leucocytoses

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Summary

Thymus-deprived mice are unable to react with eosinophilia to an appropriate stimulus, but their ability to mount a neutrophil leucocytosis in response to pyogenic infection is unimpaired. It appears that thymus-processed lymphocytes are required for induction of eosinophil but not of neutrophil leucocytosis.

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Introduction

Though eosinophilia is frequent in such conditions as parasitic infections and allergy the function of these cells and the steps leading to their increased production in man have not been established. However, in rats given *Trichinella spiralis* larvae eosinophilia has been shown to depend on a population of competent lymphocytes and to have some characteristics of an immune reaction (Basten and Beeson, 1970). The response is quantitated by measuring the blood eosinophil level, which has proved to be an accurate indicator of increased production of eosinophils in bone marrow (Basten *et al.*, 1970; Spry, 1970). We decided to determine whether this eosinophil response to trichinella larvae is thymus-dependent in mice and whether a neutrophil leucocytosis induced by pyogenic infection is similarly regulated.

Materials and Methods

Sixty CBA/H male mice were thymectomized at 8 weeks of age. Two weeks later they were subjected to 850 rads total body irradiation from a 220-kV x-ray machine. Within three hours 5×10^6 syngenic bone marrow cells were injected intravenously. Some of the mice were then grafted with a single lobe of CBA/H.T6T6 neonatal thymus beneath the capsule of the left kidney. About 30 animals prepared in this way are referred to as "reconstituted" mice. A further 30 mice not given thymus grafts are called "deprived." Thirty normal CBA/H male mice were also included in the experiment. In a few instances irradiated mice were left with the thymus in situ. Such animals

were indistinguishable in behaviour from reconstituted mice and are not separated from them in the results section. About 10 adult thymectomized mice were also studied. The method of preparation of the chimaeras has been published (Davies *et al.*, 1966).

Experimental Trichinosis.—The Culbertson strain of *T. spiralis*, originally obtained from the London School of Hygiene and Tropical Medicine, was maintained in rats by feeding them muscle-phase larvae by gastric tube. The methods of harvesting and counting have been described previously (Basten *et al.*, 1970). The stimulus for an eosinophil reaction was provided by intravenous injection of 2,500 living larvae.

Experimental Pyogenic Infection (Pyelonephritis).—Pyelonephritis was induced in mice after the method of Guze and Beeson (1956) in rats by ligating one ureter and injecting between 2 and 7×10^7 *Escherichia coli* organisms intravenously 24 hours later.

Peripheral Blood Counts.—Direct eosinophil counts were performed as described by Basten *et al.* (1970). Neutrophil counts were calculated from total white cells enumerated in a haemocytometer chamber after 1:20 dilution with fluid containing methyl green and two volumes per cent. of acetic acid, and from differential counts of 300 cells on Giemsa-stained blood smears.

Student's *t* test was used for statistical comparisons between groups.

Results

Eosinophil Response to Larvae.—In normal mice on first challenge with trichinella larvae there was a rise in the number of eosinophils to about 200 cells/mm³ (Fig. 1). When rechallenged they showed a greater eosinophilia, with a peak of over 500 cells/mm³. The reconstituted mice reacted similarly. The eosinophil response in deprived mice differed in that there was only slight eosinophilia, even after the second injection of larvae. The differences between the deprived and reconstituted groups in their responses to both first and second injections was significant ($P < 0.05$ on days four to eight after injection on both occasions). The mice which underwent adult thymectomy without irradiation showed an eosinophil response similar to the normal controls when challenged 30 days after operation. Thus the poor responses in deprived mice could not be imputed simply to absence of the thymus but rather to paucity of thymus-processed lymphocytes (T cells) as a consequence of irradiation in the absence of the thymus.

FIG. 1—Peripheral blood eosinophil responses (± 1 standard error of the mean) to first and second intravenous injections of *T. spiralis* larvae in normal (●—●), deprived (o---o), and reconstituted (x—x) mice (see text).

Neutrophil Response to Acute Pyelonephritis.—Normal and reconstituted mice showed a similar neutrophil response, but the deprived group had a higher resting neutrophil count which rose more rapidly to reach a maximum on the fourth day, at a time when the counts of the other groups were still increasing (Fig. 2). Bone marrow examinations of several mice supported the assumption that the peripheral blood neutrophilia occurring some days after the initiation of infection is the result of increased production and not merely redistribution of cells from bone marrow or marginated pool.

FIG. 2—Peripheral blood neutrophil response (± 1 standard error of the mean) to experimental pyelonephritis in normal (●—●), deprived (o---o), and reconstituted (x—x) mice.

Discussion

Though both eosinophils and neutrophils are regarded as members of the granulocytic series of blood leucocytes they exhibit notable differences in their responses to certain stimuli. Neutrophil production usually increases during bacterial infection and after administration of corticosteroid hormones, whereas eosinophil levels decrease in these situations (Opie, 1904; Gordon, 1955). Circulating eosinophils are likely to increase in number in allergic states or parasitic infestations, while the number of circulating neutrophils does not rise appreciably. The experiments reported here show another difference in that increased production of eosinophils depends on the influence of the thymus, unlike increased neutrophil production, where no such relationship could be shown.

The influence of the thymus in eosinophilia appears to be exerted through the T cell rather than through non-specific supportive or endocrine functions. Eosinophilia was not effectively diminished in adult mice 30 days after thymectomy without irradiation to remove the T-cell population. The exact role of these cells in increased production of eosinophils remains to be elucidated. Lymphocytes may stimulate bone marrow eosinopoiesis by release of a diffusible agent (Basten and Beeson, 1970), or they may play a part in the elaboration of a factor chemotactic to eosinophils. Recently lymph node cells from guinea-pigs incubated with antigen in vitro have been shown to release a substance which reacts with immune complexes to produce such an agent (Cohen and Ward, 1971). The finding that the resting level of eosinophils in deprived and normal mice is similar implies that the thymic influence comes into play only when increased production of eosinophils is required.

Mechanisms for Eosinophils

The neutrophil response to acute pyelonephritis in mice deprived of T cells was more rapid than in normal mice. Also noteworthy was their somewhat higher resting neutrophil count. This is unlikely to have been the result of irradiation, as the reconstituted mice had counts similar to the unirradiated normal group. Perhaps it is a reflection of altered reactivity to bacterial products in the immunologically deficient deprived animals. The findings in our experiments with pyelonephritis seem in agreement with other work on the Nude strain of mice, which have features of thymus deficiency (Wortis, 1971). Such mice are capable of mounting a neutrophil leucocytosis, but not a lymphocytosis, in response to challenge with *Haemophilus pertussis*.

These experiments show that increased eosinophil production in mice requires the participation of T cells. This is of particular interest in relation to a report that eosinophilia occurred after fetal thymus grafting of an infant with thymic aplasia (Cleveland

et al., 1968). No evidence was found to suggest that neutrophil leucocytosis is dependent on an intact lymphoid system, illustrating yet another difference between these two cell types.

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Trimethoprim-sulphamethoxazole in Acute Brucellosis

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Summary

Eight patients with proved brucella infection were treated with trimethoprim-sulphamethoxazole. The dose varied from two to four tablets given twice daily for three weeks. Clinical response was rapid and all patients were asymptomatic and afebrile within two to seven days of starting therapy. Three patients relapsed clinically and bacteriologically within three weeks of ending treatment. There were no side effects of the treatment. It is suggested that the treatment be continued for at least six weeks to prevent relapses.

Introduction

We (Farid *et al.*, 1970) noted the remarkable effectiveness of trimethoprim-sulphamethoxazole (Septrin, Bactrim) in the treatment of acute typhoid and paratyphoid fevers and also in two patients with proved brucella septicaemia. Simultaneously Lal *et al.* (1970), from the United Kingdom, reported the successful use of this treatment in three out of four patients with acute brucellosis and emphasized that their patients were asymptomatic and afebrile within 24 to 48 hours of starting treatment. We report here on the treatment of an additional eight patients with acute *Brucella melitensis* infection with trimethoprim-sulphamethoxazole.

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Patients and Methods

Six out of the eight patients treated belonged to one family—father, mother, three sons, and one daughter—the seventh patient was the brother of the father. All lived in Cairo, owned goats and cattle, and drank raw milk. They fell ill at about the same time during the period December 1970 to February 1971. The eighth patient was a farmer from a village near Cairo. The eight patients, six males and two females, were aged 8 to 62 years. All were acutely ill and complained of an afternoon rise of temperature accompanied by profuse sweating, with muscle and joint aches and pains. The family of six and the brother gave a short history of one to four weeks of illness; the farmer gave a history of recurrent febrile attacks for eight weeks. All had raised brucella tube agglutination titres and positive blood cultures for *Br. melitensis*.

Blood cultures were made on double-phase Castaneda (1947) bottles. Treatment was standardized at a dose of 10 mg of trimethoprim and 50 mg of sulphamethoxazole per kg per day. (Each tablet contains trimethoprim 80 mg and sulphamethoxazole 400 mg; a ratio of 1 to 5.) The total daily dose was divided into two portions given 12 hours apart. Treatment was given for three weeks. The youngest patient received two tablets twice daily and the older men received up to four tablets twice daily. All were kept in hospital for at least four weeks after completing treatment and were then followed up as outpatients for as long as possible.

Results

The results are summarized in the Table. All patients were asymptomatic within two to four days of starting treatment; headache, profuse sweating, myalgia, and arthralgia were rapidly relieved. The return of temperature to normal was somewhat slower and took from two to seven days. All patients tolerated medication very well and there were no side effects.

Three patients (the middle son, younger son, and the farmer) relapsed one to three weeks after ending treatment; all became symptomatic and had positive blood cultures for *Br. melitensis*. The farmer was re-treated with tetracycline and streptomycin because of suspected brucella spondylitis (Farid and Omar, 1965). The middle and younger sons were successfully re-treated with trimethoprim-sulphamethoxazole, but this time the course of treatment was extended to six weeks.

Discussion

There is little doubt that trimethoprim-sulphamethoxazole