

## Alterations in the T-Lymphocyte Subpopulation in Patients with Rhinoscleroma

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**T-lymphocyte subpopulations were studied in a group of patients with rhinoscleroma due to *Klebsiella rhinoscleromatis*. The data demonstrated that these patients had a significantly greater number of T-suppressor/cytotoxic lymphocytes than did clinically healthy individuals. This finding correlated with a diminished response to the T-cell mitogen concanavalin A. The evidence indicated that the T-cell response of these patients was decreased and may reflect the host's response to the bacterial invader, thus explaining the chronicity of the disease.**

Rhinoscleroma is a progressive, granulomatous infectious disease, usually limited to the upper respiratory tract, induced by *Klebsiella rhinoscleromatis* (5, 8). Epidemiologic studies have shown that the disease affects men and women equally, with the age group 15 to 35 years having the highest incidence. It appears to be transmitted only after prolonged exposure, for example, among family members living together in close quarters (23). The disease carries a high morbidity and is endemic in some parts of the world, such as eastern Europe, north and central Africa, southern Asia, the Middle East, and some regions of South and Central America. It is not distributed uniformly among populations but, instead, seems to be centered in rural areas among less-hygienic inhabitants (13). Immunological studies have been conducted to establish whether there is an adequate humoral and cellular immune response of the host against the causal organism. Serological studies provide strong evidence for the presence of humoral antibodies directed against several mucopolysaccharide fractions of *K. rhinoscleromatis*, although they are not specific enough to be used in the routine diagnosis of florid scleroma (22, 23, 25, 27). In addition, the serum from some patients with active rhinoscleroma had poor in vitro bactericidal activity against *K. rhinoscleromatis* (15). Cell-mediated immunity (CMI) in this disease has been scantily studied. A skin window test was used to examine local inflammatory reactions and the state of immunity in, as well as contacts with, patients with rhinoscleroma (26). These studies showed a deficient cellular immune response to *Klebsiella* antigen in these individuals. Another report (14) indicated the presence of T-lymphocyte subpopulations in the nasopharyngeal tissue of patients with rhinoscleroma; the investigators demonstrated that both the helper and inducer T (CD4) cells and the suppressor and cytotoxic (CD8) cells were diffusely distributed among the histiocytes without any discernible mantle.

To further investigate CMI in patients with rhinoscleroma, we studied their T-lymphocyte subpopulations, particularly the T-cell response to the mitogen concanavalin A (ConA). The evidence showed that the T-cell response of these patients was diminished; this diminished response was probably due to an increase in CD8 cells.

Patients were chosen from the Instituto Nacional de Enfermedades Respiratorias and the Hospital Central Militar

in México City. The group included 22 patients with rhinoscleroma confirmed clinically, pathologically, and bacteriologically, with an evolution ranging from 3 to 15 years; therefore, they were in either the catharral, the granulomatous, or the fibrotic stage of the disease. Ages ranged from 19 to 35 years. At the time of this study, all patients were under treatment or had received treatment with tetracycline and streptomycin, but none had received immunosuppressors. A control group was also included; it consisted of 18 healthy individuals in the same socioeconomic level and age group.

Mononuclear cells were obtained from peripheral blood by the Ficoll-Hypaque method (2). The band of mononuclear cells was recovered from the Ficoll-Hypaque interface, washed three times with balanced salt solution (centrifuged at approximately  $100 \times g$  to remove platelets), and suspended in RPMI 1640 supplemented with 5% fetal bovine serum, and the lymphocytes were counted.

The total number of lymphocytes was determined as described elsewhere (6). The results are expressed as the mean number of lymphocytes per microliter  $\pm$  standard deviation.

Lymphocytes with surface receptors for sheep erythrocytes (E) were identified by the method of Kaplan and Clark (10). Briefly, to 0.25 ml of a lymphocyte suspension ( $2 \times 10^6$  cells per ml) in supplemented RPMI 1640, 0.25 ml of a 2% suspension of E in balanced salt solution was added. The suspension was mixed thoroughly, incubated at 37°C for 15 min, and then centrifuged at  $25 \times g$  for 5 min and incubated overnight at 4°C for maximal rosette formation. The number of E rosettes was estimated by counting those lymphocytes having three or more E cells. All nonrosetting lymphocytes were also identified and counted.

Phenotyping of the lymphocytes was carried out with monoclonal antibodies OKT4 and OKT8. In brief, the cells ( $10^6$  per sample) were washed twice in phosphate-buffered saline, pH 7.2, supplemented with 2% fetal bovine serum, and suspended in an appropriately diluted sample of fluoresceinated monoclonal antibody OKT4 or OKT8 (Ortho Pharmaceutical Corporation, Raritan, N.J.). The cells were incubated for 30 min at 4°C, washed three times in phosphate-buffered saline, and analyzed on a fluorescence microscope.

Cellular proliferation was assayed by culturing  $2 \times 10^5$  peripheral blood mononuclear cells in 200  $\mu$ l of supplemented RPMI 1640 in flat-bottom microtiter wells (model no.

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TABLE 1. Determination of T-lymphocyte subpopulations in patients with rhinoscleroma and in healthy individuals

| Subpopulation     | Total % of lymphocytes<br>(mean $\pm$ SD) in: |                  | P      |
|-------------------|---|------------------|--------|
|                   | Patients with<br>rhinoscleroma                | Healthy controls |        |
| E-rosetting cells | 52 $\pm$ 1.60                                 | 59 $\pm$ 1.59    | >0.05  |
| CD8               | 33 $\pm$ 2.69                                 | 22 $\pm$ 1.23    | <0.002 |
| CD4               | 27 $\pm$ 1.92                                 | 33 $\pm$ 1.75    | >0.05  |

3596; Costar, Cambridge, Mass.) for 3 days with different concentrations of ConA, ranging from 1.0 to 100  $\mu$ g/ml, as previously described (19). All cultures were performed at least in triplicate. One microcurie of [ $^3$ H]thymidine (5.0 Ci/mmol; New England Nuclear, Boston, Mass.) was added to each well 28 h before termination of cultures. Cells were harvested with a cell harvester (Brandell, Rockville, Md.), and the glass fiber mats were counted in a liquid scintillation counter. Data are presented as mean counts per minute  $\pm$  standard deviation.

Fisher's test was used to determine the homogeneity or heterogeneity of the variances among groups (4). The significance of the difference among the means of the groups with homogeneous variances was determined by Student's *t* test; that among the heterogeneous variances was determined by the Mann-Whitney *U* test (12).

The absolute count of lymphocytes was used to determine differences, if any, between the groups under investigation. Absolute counts of lymphocytes tended to be lower in the blood of patients with rhinoscleroma than in the blood of healthy individuals; however, the difference was not significant ( $P > 0.05$ ). The number of lymphocytes per microliter was  $1,399 \pm 113$  in the patients with rhinoscleroma and  $1,519 \pm 128$  in the healthy individuals.

To determine the number of T lymphocytes present in the peripheral blood of the patients under study, the number of E-rosetting cells, as well as of CD4 and CD8 lymphocytes, was estimated. The results indicated that the number of E-rosetting cells, as well as of CD4 lymphocytes, was similar in the rhinoscleroma and control groups (Table 1). However, significant differences ( $P < 0.002$ ) were observed among the rhinoscleroma and control groups when the number of CD8 cells was determined. Patients with rhinoscleroma displayed a CD4:CD8 ratio of  $0.84 \pm 0.04$ , whereas in healthy subjects the CD4:CD8 ratio was  $1.40 \pm 0.07$ , clearly indicating a higher proportion of CD8 lymphocytes in the former group tested.

In addition, experiments were performed to determine the ability of T cells to respond to different concentrations of ConA. Lymphocytes from the patients under study reacted differently to the mitogen compared with those from healthy individuals (Fig. 1). The data show that in both groups the optimal response was observed at the dose of 10  $\mu$ g/ml and that, at this ConA concentration, the proliferative response was significantly lower in the patients with scleroma than it was in the control group ( $P < 0.005$ ). However, the data also showed that higher proliferative responses were obtained in the rhinoscleroma group than in healthy controls when doses of 100  $\mu$ g/ml were used; the difference was also significant ( $P < 0.002$ ).

The present data show an abnormality in the T-lymphocyte population of patients with rhinoscleroma. The irregularity consisted of an increased number of CD8 cells as well as a low response to a mitogen specific for T cells, namely

ConA (17), as compared with healthy individuals. It is interesting to note the proliferative response of the rhinoscleroma group to the highest dose of ConA. It is known that high concentrations of ConA induce suppressor cells (7); therefore, at this high dose of the mitogen, the suppressor cells that appeared in higher proportion in the patients with rhinoscleroma may have proliferated optimally, giving the highest [ $^3$ H]thymidine incorporation. The diminished response to the T-cell mitogen is particularly important since it indicates an abnormal CMI, which may prevent patients from eliminating the bacteria.

In studies performed with other infectious diseases produced by intracellular microorganisms, such as leprosy, similar findings have been reported (3). Thus, patients with lepromatous leprosy have a depressed cellular immune capacity to respond to antigens from *Mycobacterium leprae*, a reduction in the number of effector and CD4 cells, an increase in the number of CD8 cells (1, 24), and a reduction in the synthesis of interleukin-2 (9). Furthermore, the lymphocytes of individuals with leprosy suppressed the response to ConA of lymphocytes from healthy individuals (3), a finding that has been observed in infections caused by other acid-fast bacilli, namely *Nocardia brasiliensis* (18). The observations described above indicate the important role that CD8 cells play in regulating the immune response of individuals infected with intracellular microorganisms. In this regard, it is known that immune homeostasis results from a delicate balance of inducer and suppressor subsets within the human T-cell circuit. Perturbation in subset dynamics may initiate a variety of immunopathological disorders (20). The changes in the T-cell populations reported here may help to explain some of the pathology observed in patients with rhinoscleroma.

The granuloma particular to patients with rhinoscleroma is similar to that observed in lepromatous leprosy and is characterized by the presence of histiocytes (14). It has been postulated that the intimate contact of both helper and suppressor cells with mononuclear phagocytes may impede the immune response in patients with rhinoscleroma, thereby preventing the maturation of histiocytes into bacteriolytic epithelioid cells and allowing florid bacillary proliferation and extensive nasopharyngeal tissue destruction

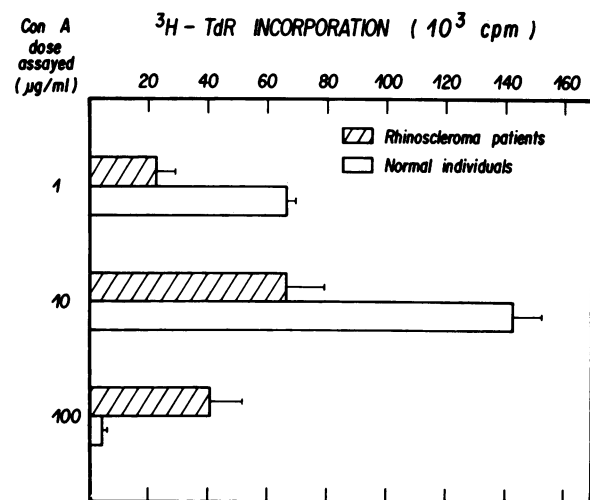


FIG. 1. ConA response of peripheral blood mononuclear cells obtained from patients with rhinoscleroma and healthy individuals. Results are expressed as the counts per minute of quadruplicate cultures  $\pm$  the standard deviation.  $^3$ H-TdR, [ $^3$ H]thymidine.

(21). Our data showing an altered ratio in the lymphocytes engaged in CMI may explain the chronicity of the disease due to the inability of the infected individuals to eliminate the microorganism. The increased number of CD8 cells may result in the inability of the infected individuals to activate the mononuclear phagocytic system (11). In infections, the activity of this accessory phagocytic system must be heightened. T-cell products secreted after immune recognition cooperate to effect this heightened activity. Such products promote increased bone marrow activity and resultant production of macrophages, and they also cause a heightened functional activity in the final effector cells (16).

The heterogeneity of the results obtained in the patients with rhinoscleroma reported here is very probably due to the different stages of evolution of the disease, which in the present study varied from 3 to 15 years. Nevertheless, the results pointed to a modification in the T-cell population.

In summary, in this investigation we found that patients with rhinoscleroma showed an alteration in the ratio of CD4:CD8 cell subsets and an abnormal proliferative response to ConA, which may reflect the host's response to the bacterial invader and help to explain the chronicity of the disease.

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