

B Lymphocyte Colony Formation in Renal Infection

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The effect of renal infection on B lymphocyte colony formation has been investigated in the belief that a study of the effect of infection on subpopulations of lymphoid cells might provide direct evidence of the effect of infection on the immune system. Renal infection was induced in mice, and the specific immune response of the B lymphocyte compartment was quantitated by determining the serum antibody and plaque-forming cell response to infection. Under the conditions of the experiment, the ability of splenic lymphocytes to form B lymphocyte colonies was significantly depressed during the first 7 days of infection, and the results suggest that a study of the responses of lymphoid cells to infection may provide information of diagnostic and prognostic value.

Several attempts have been made to study the effects of infection on B and T lymphocyte populations, but the results have not provided as much additional information of diagnostic or prognostic value as might have been expected. Recently, semisolid culture systems have been developed in which B lymphocytes undergo cell division to form readily visible colonies (7, 8). Such cloning systems have made it possible to analyze additional aspects of the cellular response of the host to an immunogenic challenge. Although the cloning of progenitor cells offers many advantages in the analysis of the response of B and T lymphocyte populations to antigenic stimulation, the procedure has not been utilized to study the immunobiology of an infectious disease.

In the present experiments we have investigated the effect of renal infection on B lymphocyte colony formation in the belief that a study of the effect of an infection on the subpopulation of lymphoid cells forming B cell colonies might provide more direct evidence of the effect of infection on the immune system than the study of changes in the ratio of peripheral blood lymphocytes.

MATERIALS AND METHODS

Animals. Adult CBA mice maintained as an inbred strain were used in these experiments.

Experimental renal infection. The strain of *Escherichia coli* used to induce infection was the same as that used in previous studies of experimental pyelonephritis (10, 11, 13). A total of 3×10^8 microorganisms were introduced at three sites in the surgically exposed kidney, using a fine glass capillary (12).

Bacteriological examination. Nutrient agar pour plates of serial 10-fold dilutions of homogenized kidney were used to obtain the bacterial count per gram of wet renal tissue.

Histological processing. Tissue for histological examination was placed directly into a 0.1% solution of cetylpyridinium chloride in 10% formaldehyde. After routine processing and cutting, sections were stained with hematoxylin and eosin.

B lymphocyte culture. The cultural procedure was essentially as described by Metcalf et al. (6-8). The spleens were removed under sterile conditions, and dispersed cell suspensions prepared by teasing the tissue in HEPES (*N*-2-hydroxyethyl piperazine-*N'*-2-ethanesulfonic acid)-buffered minimum essential medium. A 4-ml amount of the cell suspension was layered over 1 ml of fetal calf serum and allowed to stand for 5 min. The cell suspension above the fetal calf serum layer was pipetted into a further tube and washed once with the HEPES minimal essential medium by centrifuging at $400 \times g$ for 5 min. For each culture, 1×10^6 splenic lymphocytes were resuspended in 2.5 ml of tissue culture medium, and 0.25 ml of a 10% sheep erythrocyte (SRBC) suspension was added. This suspension was then added to 2.5 ml of 1% agar maintained at 42°C. Cultures (1 ml each) of the semisolid culture medium were prepared in 35-mm plastic petri dishes (Falcon Plastics) and the cultures held in a humidified incubator in an atmosphere of 10% CO₂ in air. After incubation for 6 days, the cultures were flooded with 0.5 ml of 3% acetic acid to allow visualization. Colonies, defined as discrete aggregates of 50 or more cells, were clearly visible and were scored by using a standard Leitz microscope at $\times 35$ magnification.

Determination of serum antibody response to *E. coli*. Blood was collected by heart puncture from mice with experimentally induced renal infection over a 21-day period. After separation from the erythrocytes, the serum was stored at -20°C until analyzed. Serum antibody levels were determined by using a passive hemagglutination procedure previously described (3).

Plaque-forming cells producing specific antibody against the somatic antigens of *E. coli* O75. SRBC were sensitized with Boivin-type endotoxin prepared by trichloroacetic acid extraction of a culture of

E. coli O75 essentially as described by Webster and co-workers (17). Activation of the endotoxin preparation was carried out by heating the endotoxin in an alkaline medium (4). A solution of endotoxin, 0.12 mg/ml, was prepared in 0.02 N NaOH and heated for 5 min in a boiling water bath. On cooling, the pH was adjusted to 7, and an equal volume of 0.3 M phosphate-buffered saline was added. This preparation was stable and maintained its activity after several weeks of storage at 4°C. To sensitize the SRBC, equal volumes of washed SRBC and activated endotoxin were mixed and incubated at 37°C for 2 h, then washed three times with phosphate-buffered saline. Sensitization of SRBC with the endotoxin was confirmed by carrying out passive hemagglutination tests with an anti-*E. coli* O75 antiserum standardized against a bacterial antigen. The plaque assay system used was a modification of the Jerne plaque assay described by Cunningham and Szenberg (2), and the specificity of the antibody response against the somatic antigens of the infecting *E. coli* strain was determined by carrying out duplicate assays, using antigen sensitized and nonsensitized SRBC. Plaques appearing against the unsensitized SRBC were subtracted.

Experimental design. Details of the model of experimental renal infection were first established in mice. An immune response to renal infection in these animals was confirmed by quantitating the number of antibody-forming cells and demonstrating a serum antibody response to the challenging microorganism. The effect of infection on the formation of B lymphocyte colony numbers was then investigated.

RESULTS AND DISCUSSION

Pyelonephritis. Details of the model in the mouse were established in an independent series of experiments before commencing these stud-

ies. A total of 23 animals were challenged, and all showed gross pathological and bacteriological evidence of renal infection (Fig. 1). Histopathological changes consistent with the induction of acute renal infection were also found, and wedge-shaped lesions were seen that initially contained polymorphonuclear leukocytes that were later replaced with a lymphocytic infiltrate. The results were representative of the pathological changes found in the pyelonephritic mice used through out these experiments.

Plaque-forming cell and serum antibody response against the infecting organism. A methodological check of the assay system was carried out by determining the splenic response of mice to an intravenous challenge of SRBC. After this challenge, plaque-forming cells were first detected 3 days after stimulation and reached a peak 4 days after challenge. Pyelonephritis was then produced in 39 mice, and the animals were killed at intervals up to 14 days after challenge. An increase in the number of splenic lymphocytes synthesizing specific antibody against the infecting organism was first detected 3 days after challenge, and a 500-fold increase in the number of plaque-forming cells was demonstrable 14 days after challenge. Renal infection was then established in 32 mice, and 8 mice from the group were bled by heart puncture at four separate intervals. The titer of specific serum antibody against the infecting *E. coli* strain (*E. coli* O75) increased steadily over the 14-day interval studied (Table 1). Titers (mean \log_2 for six mice) of endotoxin-treated SRBC

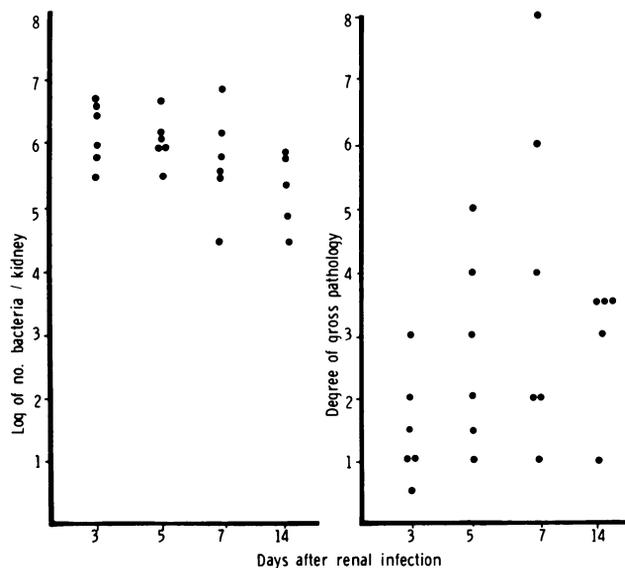


FIG. 1. The gross pathological and bacteriological features of experimentally induced renal infection 3, 5, 7, and 14 days after the initiation of infection. On the scale used to report the gross pathological changes, a 0 to 10 scale was used, with each unit of the scale representing one-tenth of the surface of the kidney.

TABLE 1. Quantitation of plaque-forming cells (PFC) synthesizing antibacterial antibody in animals with experimentally induced *E. coli* pyelonephritis

Days after challenge	No. of mice	Background PFC/spleen (SRBC)	Total PFC/spleen (Endo-SRBC)
0	5	50	60
3	6	254	1,405
5	5	407	11,724
7	13	180	3,391
14	11	330	37,140

were 1, 2.2, 3.5, 3.8, and 7.3 on days 0, 3, 5, 7, and 14, respectively. All corresponding titers of untreated SRBC were <1.

B lymphocyte colony formation. Splenic lymphocytes, cultured in the presence of mercaptoethanol and *E. coli* lipopolysaccharide, developed colonies with the same gross morphology and dependence on accessory factors as the B lymphocyte colonies originally described and characterized by Metcalf et al. (7, 8). A photograph of a typical B lymphocyte colony is shown in Fig. 2. A linear response was found over a

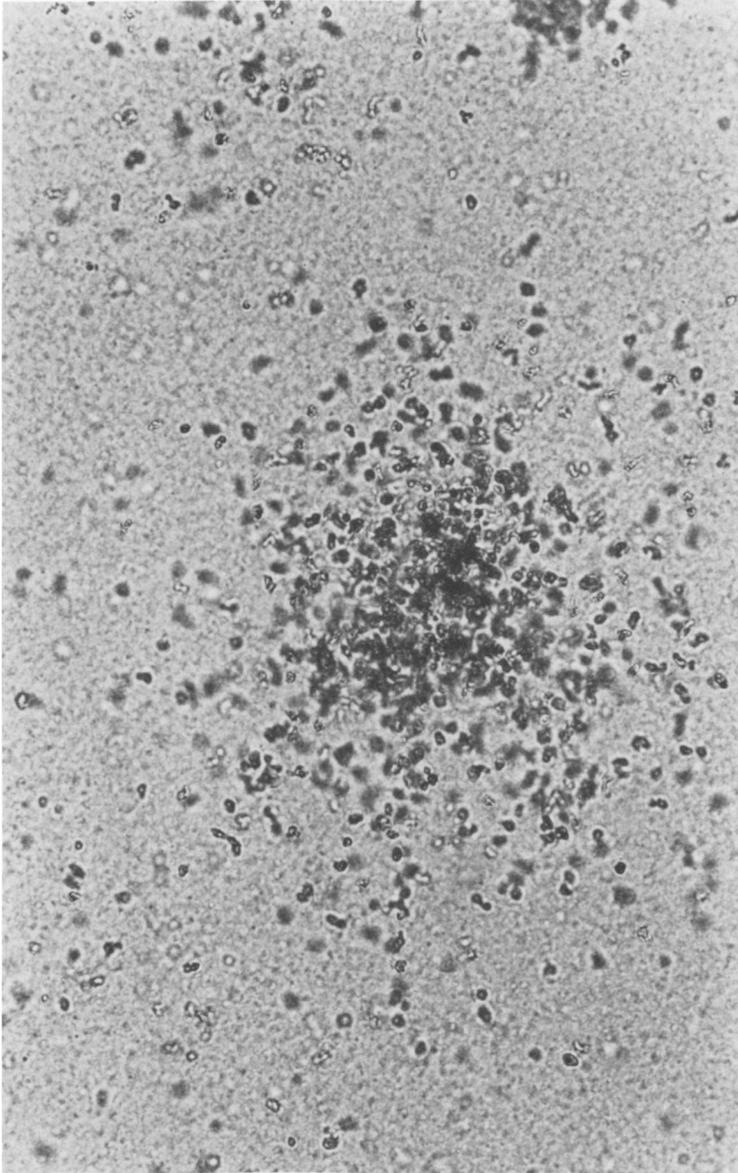


FIG. 2. A 7-day culture of CBA splenic lymphocytes. B lymphocytes have proliferated to form a colony of 50 or more cells. Colonies often contained a central region of tightly packed cells and a loose outer mantle of cells.

fourfold range of splenic lymphocyte concentrations from both normal and pyelonephritic animals, and in the experimental analyses that followed, a single concentration of splenic lymphocytes was cultured.

Effect of infection on B lymphocyte colony formation. Unilateral pyelonephritis was induced in a total of 38 animals, and cultures of splenic lymphocytes were carried out 1, 3, 5, 7, and 14 days after challenge. At the time of death, splenic lymphocyte suspensions were prepared from pyelonephritic and counterpart normal animals. In each experiment, the number of B lymphocyte colonies cultured from splenic lymphocyte suspensions from pyelonephritic animals was expressed as a percentage of the mean number of colonies cultured from splenic lymphocyte suspensions of normal animals examined at the same time (mean of 4,802 colonies per 10^6 lymphocytes from the 32 normal animals cultured). The number of B lymphocyte colonies cultured from mice with acute renal infection was found to be significantly depressed (Student's *t* test) during the first 7 days of infection, but had returned to normal 14 days after challenge (Fig. 3). Recent advances in lymphocyte physiology have presented an opportunity to explore alterations in the normal ratio of lymphocyte subpopulations as a potential diagnostic and prognostic aid in the management of infec-

tious diseases. Several clinical studies have already investigated the effect of infectious diseases on B and T lymphocytes in peripheral blood, but with varying results. One report claimed that there was no change in the number of circulating B and T lymphocytes (1), whereas other studies (14, 16) have observed serial changes in the relative proportions of T and B lymphocytes during the course of acute infections. Although current methods of identification allow quantitative analyses of T and B lymphocyte numbers to be made, the availability of functional tests of B lymphocyte activity would be considered a major advance. The development of *in vitro* methods for the culture of murine B lymphocytes has raised the possibility that this characteristic might ultimately serve as an analysis for B lymphocyte function in humans.

The present study has established that acute pyelonephritis developed consistently in the experimental animal model and that the hosts B lymphocytes responded to infection by synthesizing specific antibacterial antibody. As a subpopulation of B cells, B lymphocyte colony-forming cells might also have been expected to show a response to antigenic challenge but under the conditions of the experiment the colony-forming ability of splenic B lymphocytes was in fact depressed. The reason for the reduced abil-

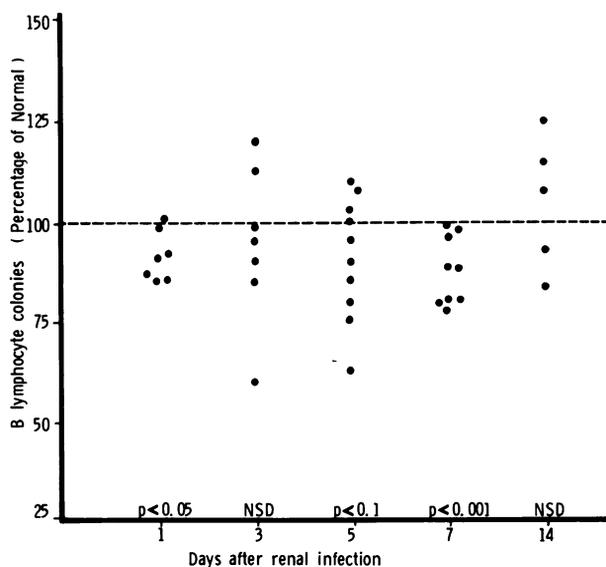


FIG. 3. Effect of renal infection on the development of B lymphocyte colonies. In each experiment, splenic lymphocyte suspensions were prepared from pyelonephritic and normal animals. The number of colonies developing from splenic lymphocyte suspensions was expressed as a percentage of the mean number of colonies cultured from splenic lymphocyte suspensions of normal animals cultured at the same time (mean of 4,802 colonies per 10^6 lymphocytes from the 32 normal animals examined). Statistical analysis was by Student's *t* test.

ity of B lymphocytes to form colonies was not clear but may be a further demonstration of the effect of renal infection on a specific immune function (9). As a functional test, however, the assay does promise to provide evidence of an immune system challenged with an infectious agent.

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LITERATURE CITED

1. Aiuti, F., M. V. Ciarla, C. D'Asero, R. D'Amelio, and J. A. Garofalo. 1973. Surface markers on lymphocytes of patients with infectious diseases. *Infect. Immun.* **8**: 110-117.
2. Cunningham, A. J., and A. Szenberg. 1968. Further improvement in the plaque technique for detecting single antibody-forming cells. *Immunology* **14**:599-600.
3. Douglas, R., T. E. Miller, and L. Scott, 1974. Estimates of the immune response to bacterial antigen by means of haemagglutinating autoanalyser. *J. Infect. Dis.* **130**: 651-655.
4. Landy, M., R. J. Trapani, and W. R. Clark. 1955. Studies on the O antigen of *Salmonella typhosa*. III. Activity of the isolated antigen in the haemagglutination procedure. *Am. J. Hygiene* **62**:54-65.
5. Mackowiak, P. A. 1978. Microbial synergism in human infections. *N. Engl. J. Med.* **298**:21-26 and 83-87.
6. Metcalf, D. 1976. Role of mercaptoethanol and endotoxin in stimulating B lymphocyte colony formation in vitro. *J. Immunol.* **116**:635-638.
7. Metcalf, D., G. J. V. Nossal, N. L. Warner, J. F. A. P. Miller, T. E. Mandel, J. E. Layton, and G. A. Gutman. 1975. Growth of B-lymphocyte colonies in vitro. *J. Exp. Med.* **142**:1534-1549.
8. Metcalf, D., N. L. Warner, G. J. V. Nossal, J. F. A. P. Miller, and K. Shortman. 1975. Growth of B lymphocyte colonies in vitro from mouse lymphoid organs. *Nature (London)* **255**:630-631.
9. Miller, T., L. Scott, E. Stewart, and D. North. 1978. Modification by suppressor cells and serum factors of the cell-mediated immune response in experimental pyelonephritis. *J. Clin. Invest.* **61**:946-972.
10. Miller, T. E., S. Burnham, and J. D. K. North. 1976. Immunologic enhancement in the pathogenesis of renal infection. *Clin. Exp. Immunol.* **24**:336-345.
11. Miller, T. E., D. Layzell, and E. Stewart. 1976. Experimental pyelonephritis: The effect of chronic active pyelonephritis on renal function. *Kidney Int.* **9**:23-29.
12. Miller, T. E., and K. B. Robinson. 1973. Experimental pyelonephritis: a new method for inducing pyelonephritis in the rat. *J. Infect. Dis.* **127**:307-310.
13. Miller, T. E., and J. W. Smith. 1971. Antibody synthesis in kidney, spleen and lymph nodes in acute and healed pyelonephritis. *Br. J. Exp. Pathol.* **52**:678-683.
14. Niklasson, P. M., and R. C. Williams. 1974. Studies of peripheral blood T and B-lymphocytes in acute infections. *Infect. Immun.* **9**:1-7.
15. Stobo, J., S. Paul, R. E. Van Scoy, and P. E. Hermans. 1976. Suppressor thymus-derived lymphocytes in fungal infection. *J. Clin. Invest.* **57**:319-328.
16. Thorley, J. D., J. W. Smith, J. P. Luby, and J. P. Sanford. 1977. Peripheral blood lymphocyte response to acute infections in humans. *Infect. Immun.* **16**:110-114.
17. Webster, M. E., J. F. Sagin and M. Landy. 1955. Studies on the O antigen of *Salmonella typhosa*. Purification of the antigen. *J. Immunol.* **74**:455-465.