Modification by Suppressor Cells and Serum Factors of the Cell-Mediated Immune Response in Experimental Pyelonephritis

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ABSTRACT A marked suppression of the thymus-derived (T)-lymphocyte response to concanavalin A has been demonstrated in vitro during renal infection. Suppression of the T-lymphocyte response in vitro was seen as early as 2 h after the induction of renal infection, but maximum suppression was found 24–72 h later. A population of suppressor cells in the splenic lymphocyte population, generated during the host's response to infection, contributed to the depressed lymphocyte response. Removal of suppressor cells restored the mitogenic responsiveness of the remaining splenic lymphocytes. Conversely, in co-culture experiments, a suppressor cell present in the splenic lymphocyte population of pyelonephritic animals was shown to be capable of suppressing the mitogenic responsiveness of normal splenic lymphocytes. Significantly reduced host vs. graft responses by the pyelonephritic animals confirmed, in vivo, the depression of cell-mediated immune mechanisms.

An additional suppressive factor was found in the serum of pyelonephritic animals which depressed in vitro the mitogenic responsiveness of splenic lymphocytes from normal animals. Support for the suppressive role of this serum factor was found when splenic lymphocytes from pyelonephritic animals were tested in vivo in the absence of homolgous serum (graft vs. host). Under these conditions, the lymphocytes showed an enhanced reaction compared with lymphocytes from normal animals. The presence of a suppressor cell population and a serum factor, both capable of depressing cell-mediated mechanisms, may be major factors contributing to the establishment of infection in the kidney.

INTRODUCTION

Although the epidemiology and natural history of renal infection is well documented, convincing studies that explain the peculiar susceptibility of renal tissue to infection are lacking. Recently, a number of investigators have studied the nature of the immune response to renal infection and have characterized the biology of the host response to infectious challenge (1–8). As a result, details of the local and systemic immune response against the invading organism have been described in detail, but the biological significance and relationship of the activity of the immune system to the pathogenesis and immunobiology of pyelonephritis is still unclear.

Early studies indicated that the renal environment was not a favorable milieu for the expression of some host defense mechanisms (9) and recently it has been reported that thymus-derived (T)-lymphocyte function may be depressed in the renal environment (10, 11), which suggests an association between the persistence of infection and an impairment of cell-mediated immune responses. Other experiments have indicated that the immune response to infection not only is ineffective in eliminating the invading organism, but actually protects the organism from host defense mechanisms (12).

In the present experiments we describe the modulation of the host's immune response to renal infection coinciding with the period when rapid bacterial growth is taking place in the kidney. These studies have established that in vitro a marked suppression of T-lymphocyte response to mitogenic stimulation occurs during renal infection with *Escherichia coli* and that the functional capacity of the T-lymphocytes in vivo is also significantly reduced. An immunosuppressive material was found in the serum of the pyelonephritic animals and a population of cells capable of suppressing the splenic lymphocyte response to a T-lymphocyte mitogen was also detected. Removal of the suppressive cells restored the mitogenic responsiveness of the lymphocytes from the pyelonephritic animals to normal levels.

Received for publication 16 May 1977 and in revised form 30 November 1977.

 J. Clin. Invest. © *The American Society for Clinical Investigation, Inc., 0021-9738/78/0401-0964 $1.00*

METHODS

Animal strains. Female rats weighing 220–230 g obtained from an inbred strain of DA rats were used. Inbred rats of the HS and AS2 strains, which have a major incompatibility at the Ag-B locus, were used in the graft vs. host reaction.

Bacterial strain. The strain of *E. coli* 0.75 used in these experiments was the same as that used in previous studies of experimental pyelonephritis (5–8).

Production of renal infection. Pyelonephritis was induced by direct inoculation of *E. coli* into the surgically exposed kidney with a glass microcapillary. Details of this method have been given previously (13).

Bacterial content of renal tissue. Nutrient agar pour plates of serial 10-fold dilutions of homogenized kidneys were made to obtain the bacterial count per gram of wet renal tissue.

In vitro analysis of concanavalin A-stimulated mitogenesis of T lymphocytes. Lymphoid cells were obtained from the spleens by mincing them with curved iris scissors and the tissue fragments were teased through a 60-mesh stainless steel gauze to produce a single cell suspension. This was washed once and then placed in cold Eagle's minimal essential medium (MEM)[1] (Grand Island Biological Co., Grand Island, N. Y.), buffered with Hepes to a final pH of 7.2 (Hepes-MEM). After removing cell clumps (5 min at 1 *g*) the cell suspension was counted and sufficient cells were sampled to provide a culture in triplicate; each culture contained 5×10^6 lymphocytes. These lymphocytes were again centrifuged and then deposited cells resuspended in "insert" medium (see below).

Roswell Park Memorial Institute complete medium (RPMI-1640) was used in all the experiments and contained 70 ml of 2.8% sodium bicarbonate per liter to maintain the culture at pH 7.2 in an atmosphere of 10% CO_2 in air. The medium also contained 100 μg/ml of penicillin and 100 μg/ml of streptomycin. Rat serum obtained from control animals at the time of sacrifice was added to the complete medium to give a final concentration of 5% rat serum and this provided the "insert" medium in which the cells were cultured. 5×10^6 splenic lymphocytes in 1 ml of insert medium were suspended on a dialysis membrane in a Marbrook culture vessel (14), containing 10 ml of RPMI-1640 medium in the external reservoir. 5 mg of concanavalin A (Con A) was dissolved in 5 ml of phosphate-buffered saline and 10 μl was added to the contents of each "insert" tube. Unstimulated cultures containing splenocytes without Con A were included as a control with each cell suspension assayed. Cultures were held for 3 days at 37°C in a humid atmosphere of 10% CO_2 in air. 16 h before harvesting on the 4th day, 1 μCi of [^{3}H]thymidine (2 Ci/mmol) was added to each culture (The Radiochemical Centre, Amersham, England). The amount of [^{3}H]thymidine incorporated into cellular DNA was determined after terminating the culture by the addition of distilled water, and filtration of each culture through a Whatman GFC 2.5-cm glass fiber filter (Whatman, Inc., Clifton, N. J.) held in a machined metal block. The contents of the insert were rinsed with a further volume of distilled water and finally with 1% and 5% TCA to precipitate macromolecules retained in the fiberglass filter. Filters were transferred to glass vials for [^{3}H]thymidine counting which was carried out in a Beckman model B liquid scintillation spectrometer (Beckman Instruments, Inc., Fullerton, Calif.). The procedure gave consistent replicate values to within 10%. Con A stimulation of 5×10^6 splenocytes from normal animals resulted in a [^{3}H]thymidine incorporation of approximately 100,000 dpm/10^6 lymphocytes after the addition of 1 μCi of [^{3}H]thymidine. In unstimulated control cultures, less than 1,000 dpm/10^6 lymphocytes were incorporated.

Graft vs. host and host vs. graft response. The popliteal lymph node weight method of assay for determining the graft vs host activity of rat lymphoid cells was carried out as described by Ford et al. (15) with the F1 progeny of the Ag-B disparate rat strains AS2 and HS. 5×10^6 splenic lymphocytes from control and pyelonephritic parental animals of the AS2 strain were injected subcutaneously into the left foot pad of F1 progeny at 4–6 wk of age. A similar number of syngeneic cells were introduced into the right foot pad and in a third group, culture medium alone was injected. 7 days later the recipient animals were killed and the popliteal lymph nodes were removed and weighed after the adherent connective tissue had been removed. A similar protocol was used to determine the host vs graft response of normal and pyelonephritic animals. Pyelonephritis was induced in parental AS2 animals and 5×10^6 splenic lymphocytes from the F1 progeny were introduced into the left foot pad of pyelonephritic animals 2 days after challenge. Syngeneic lymphocytes were injected into the right foot as a control. 4 days later the parental host animals were killed and the popliteal lymph nodes removed and weighed (16).

Fractionation of splenic lymphocytes. Splenic lymphocyte suspensions were prepared in RPM1-1640 by teasing tissue fragments through a 60-mesh stainless steel gauze. Clumps were removed by allowing the suspension to stand on ice for 5 min. The splenocyte suspension was then washed once in RPM1-1640 resuspended in 10 ml of the same medium and then centrifuged at 30 *g* for 2 min (17). Deposited cells were recovered as the "pelletable fraction" and the lymphocytes in the supernate were labeled the "nonpelletable fraction."

Statistical analysis. Analysis of the data was carried out with the Wilcoxon sum of ranks method.

Procedural controls. Normal animals, subjected to a sham challenge with saline in place of the bacterial inoculum, were used as the control group in these experiments.

RESULTS

Pyelonephritis. Injection of *E. coli* into the kidney with a glass microcapillary results in a consistent and reproducible injection in the renal parenchyma. The earliest gross pathological changes are detected on the 4th day after infection as discrete, wedge-shaped cortical lesions originating along the line of inoculation and extending into the medulla of the kidney. Histological evidence of active renal infection is detected 48 h after challenge. A transient bacteremia occurs in the 30 min after inoculation of bacteria into the kidney but subsequent blood cultures are sterile. A small number of bacteria are found in the spleen and liver for 48 h after the induction of infection, whereas the bacterial content of the pyelonephritic kidney remains high. The increase in bacterial numbers in the kidney during the first 48 h after bacterial challenge is shown in Fig. 1.

In vitro response to Con A of splenic lymphocytes from pyelonephritic animals. In these experiments the ability of splenic lymphocytes to respond to Con A stimulation in culture was used as an index of their potential function as effector cells in the cell-

[1] *Abbreviations used in this paper*: Con A, concanavalin A; MEM, Eagles's minimal essential medium.

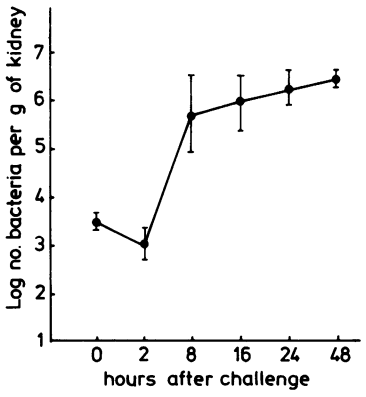

FIGURE 1 Bacterial numbers in the kidney after the direct inoculation of *E. coli* 975. Each point is the mean of the results obtained from six animals±SEM.

mediated immume response. In 55 consecutive cultures of splenocytes from normal animals an average of 119,726 dpm/10^6 lymphocytes was found after the addition of 1 μCi of tritiated thymidine to the culture. With each experiment, age-matched sham-operated animals were killed and cultures prepared in parallel with the test animals. The results (Fig. 2) are expressed as a percentage of the response of lymphocytes from normal animals. A decrease in the response of T lymphocytes in the spleen was found as early as 2 h after the initial challenge, and by 24 h a dramatic decrease in the mitogenic responsiveness of splenic lymphocytes was found which persisted for 48 h in all of the pyelonephritic animals. In 46% of the animals the ability to respond to Con A was less than 10% of normal and in 7 of 23 individual animals the response was less than 5% of normal. 7 days after challenge no difference between the response of normal and pyelonephritic animals could be demonstrated.

Suppressor cell depression of the mitogenic response. Antigen administration may result in a decreased response of rat T lymphocytes to mitogen, mediated through an antigen-activated suppressor cell (18). In the present experiments a search was made for a population of suppressor cells capable of depressing the T-lymphocyte response. Splenic lymphocyte suspensions from normal and pyelonephritic animals were fractionated into a pelletable and non-pelletable fraction. The pelletable fraction consisted of a mean of 18% of the splenic lymphocytes in the pyelonephritic animals (21 animals killed 3 days after challenge) and 16% of the splenic lymphocytes from 15 sham-operated control animals. Standard cultures in triplicate were established containing: (*a*) unfractionated splenic lymphocytes from pyelonephritic and control animals, (*b*) nonpelletable splenic lymphocytes from the supernate of the centrifuged suspensions, and (*c*) pelletable cells recovered after low-speed centrifugation. All cultures were stimulated with Con A and their mitogenic response was determined. As previ-

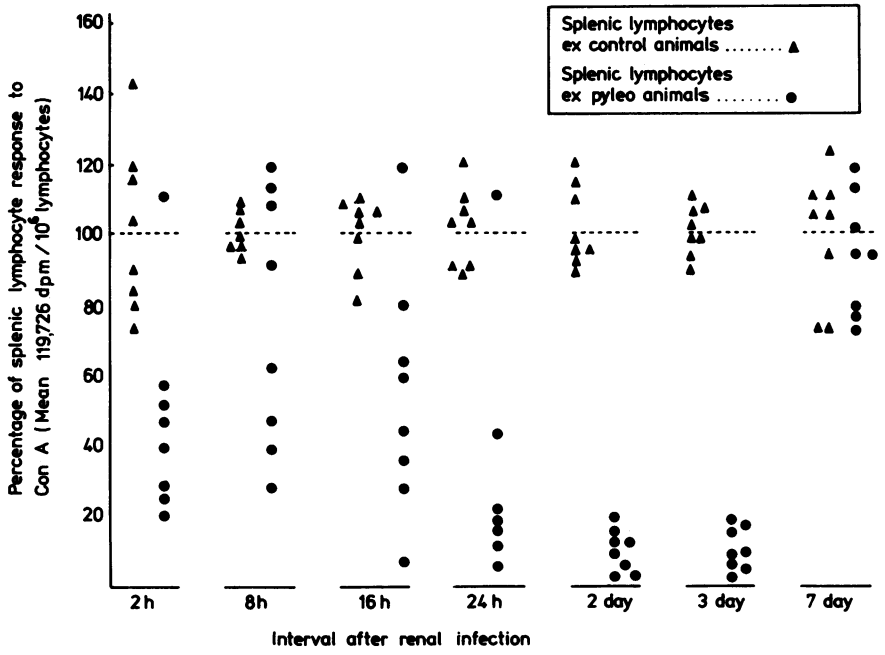

FIGURE 2 Comparative response to Con A of splenic lymphocytes from normal and pyelonephritic animals. Each point from both normal and pyelonephritic animals has been calculated and expressed as a percentage of the mean mitogenic response of all 55 normal animals which was 119,726 dpm/10^6 lymphocytes.

ously demonstrated, the response to Con A of splenic lymphocytes from pyelonephritic animals, compared with sham-operated control animals, was markedly reduced. The mitogenic responsiveness of lymphocytes from the pyelonephritic animals was restored when cells, pelletable by centrifugation at 30 *g* for 2 min, were removed from the suspension of splenocytes (Fig. 3). The response of the remaining splenic lymphocytes (82%, labeled "not pelletable" in the figure) to Con A was similar to the supernatant lymphocytes from normal animals. The pelletable fraction from sham-operated control animals was also capable of suppressing the response of normal splenic lymphocytes to Con A but to a much lesser degree.

Co-culture demonstration of a suppressor cell population. Although restoring the immune responsiveness of splenic lymphocytes by removing the suppressor cells is strong presumptive evidence for the generation of a suppressor cell population, formal proof demands that the putative suppressor cells, when examined in co-culture, be able to suppress the mitogenic responsiveness of normal splenic lymphocytes. This experiment was carried out with splenic lymphocytes from normal animals and the pelletable fraction from pyelonephritic animals 48 h after challenge. Pelletable cells obtained from splenic lymphocytes from normal animals were used as a control. The results (Table I) have shown that pelletable cells from pyelonephritic animals, when compared to pelletable cells from normal animals, can greatly suppress the Con A response of normal splenic lymphocytes. Similar suppression was seen in experiments carried out 72 h after challenge.

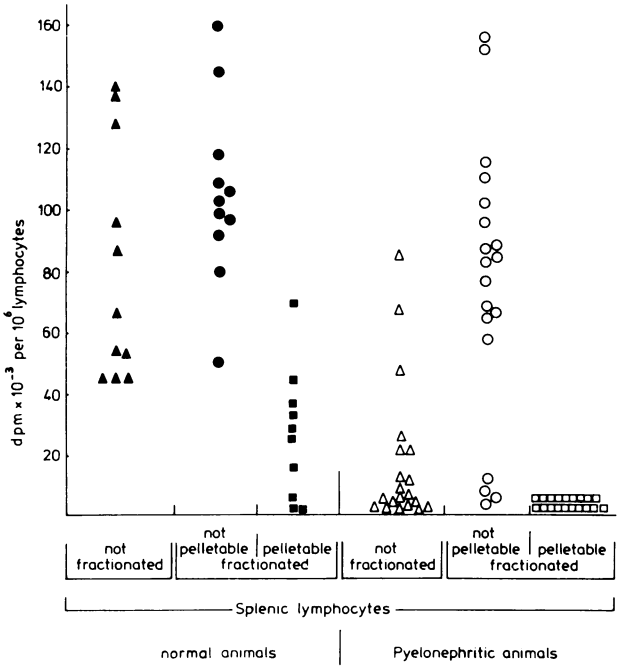

FIGURE 3 "Suppressor cell" depression of the mitogenic response. Suspensions of splenic lymphocytes from pyelonephritic animals challenged 72 h previously and control animals were prepared and separated by centrifugation at 30 *g* into a pelletable and nonpelletable fraction. The responses to Con A of fractionated and nonfractionated splenic lymphocyte suspensions were then determined. Each point is the mean of triplicate cultures.

Response of T lymphocytes in vivo (host vs. graft response). Additional experiments were carried out to assess in vivo, in the pyelonephritic animal, the functional capacity of cell-mediated immune mechanisms. Pyelonephritis was induced in a group of 10 adult animals and 48 h later 5×10^6 lymphocytes bearing an allogenic histocompatibility antigen obtained from an F1 animal were injected into the foot pads of the pyelonephritic and normal animals. Popliteal lymph nodes were removed 4 days later to determine the extent of the host vs. graft response. The cell-mediated immune responses of the pyelonephritic animals to the allogeneic histocompatibility antigens were significantly reduced compared to normal control animals ($P = 0.01$, Fig. 4).

Effect of serum from normal and pyelonephritic animals on the response of splenic lymphocytes to Con A. Having demonstrated the presence of suppressor cells both in vitro and in vivo, the possibility that serum of pyelonephritic animals might contain suppressor activity was next evaluated. Duplicate tissue cultures were established with splenic lymphocytes from normal animals with the addition of serum from normal animals or pyelonephritic animals 72 h after challenge. In this experiment two factors associated with the effect of normal and pyelonephritic serum on the responses of normal splenocytes were examined. These were: (*a*) The effect of varying concentrations of normal serum and serum from pyelonephritic animals on the response of splenic lymphocytes to Con A. (*b*) The effect of culturing splenic lymphocytes in varying concentrations of normal and pyelonephritic serum as above but delaying the addition of Con A to the splenic lymphocyte cultures for 24 h. There was a highly significant depression of lymphocyte reactivity to Con A when serum from pyelonephritic animals was added to the culture medium. However, this was demonstrable only after an interval of 24 h had elapsed between the initiation of the lymphocyte culture and the addition of the mitogen (Fig. 5A). When the Con A was added at the time the lymphocyte culture was initiated, no significant depression of mitogenic responsiveness by the pyelonephritic serum was found (Fig. 5B).

Functional activity in vivo of isolated splenic lymphocytes from pyelonephritic animals. The capacity of isolated splenic lymphocytes from pyelonephritic animals to initiate a graft vs. host response in

TABLE I

Co-Culture Demonstration of Suppressor Cell Activity in the Pelletable Fraction of Splenic Lymphocytes from Pyelonephritic Animals

Individual cultures			Co-cultures				Percent of suppression of normal response by	
Normal splenic lymphocytes	Pyelonephritic pelletable fraction	Normal pelletable fraction	Anticipated response		Observed response		Pyelonephritic pelletable fraction	Normal pelletable fraction
(A)	(B)	(C)	(A + B)	(A + C)	(A + B)	(A + C)	%	
256,220 ±16,018	334 ±156	10,800 ±3,820	256,554*	267,020	49,281	235,892	81	8
256,220 ±16,018	789 ±298	22,840 ±5,840	257,009‡	279,060	40,443 ±5,514	163,279 ±13,199	84	36
256,220 ±16,018	1,959 ±510	38,569 ±7,215	258,179§	294,789	10,597 ±5,191	207,225 ±16,200	96	19

Conditions of co-culture:

* 2.5×10^6 normal splenic lymphocytes + 0.6×10^6 pelletable cells. Mitogenic response of lymphocytes to Con A dpm/10^6 lymphocytes ± SEM.

‡ 2.5×10^6 normal splenic lymphocytes + 1.25×10^6 pelletable cells.

§ 2.5×10^6 normal splenic lymphocytes + 2.5×10^6 pelletable cells.

the absence of suppressive serum factors was then investigated.

Pyelonephritis was induced in four groups of nine animals and the ability to initiate a graft vs. host response of T lymphocytes in splenic lymphocyte preparations from pyelonephritic animals was determined. The pyelonephritic animals were studied 24 h, 2, 3, and 7 days after challenge. Splenic lymphocyte preparations from normal animals were included as controls. Lymphocytes from pyelonephritic animals 24 h after challenge had the same ability to induce a graft vs. host response as splenic lymphocytes from normal animals ($P = >0.1$). Splenic lymphocytes studied in isolation 48 h (Fig. 6) and 72 h after challenge, however, showed an enhanced graft vs. host response compared with normal splenic lymphocytes ($P = <0.01$). There were no differences in the graft vs. host response between lymphocytes taken 7 days after challenge and lymphocytes from normal animals.

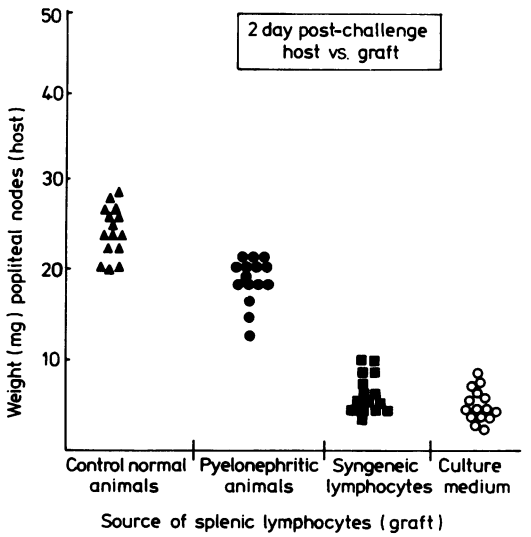

FIGURE 4 Host vs. graft analysis. 5×10^6 splenic lymphocytes obtained from F1 hybrid animals (AS2 × HS) were injected into the footpads of pyelonephritic and control animals (AS2) 48 h after renal infection was induced in the pyelonephritic animals. Popliteal lymph node weights were determined 4 days later.

Specific depression of lymphocyte function by renal infection. An inoculum of 3×10^5 viable *E. coli* was used to induce a unilateral renal infection in three animals and a deep intramuscular infection in the thigh of a further group of three animals. All animals, including a control group, were sacrificed 72 h after challenge and the response of the splenic lymphocytes to Con A was determined. The mitogenic responsiveness of splenic lymphocytes from animals with pyelonephritis was considerably reduced (Table II), whereas muscle infection initiated with a similar challenge did not have any marked effect on lymphocyte responsiveness.

DISCUSSION

A feature of this study has been the demonstration of a population of suppressor cells activated or proliferating during the early stages of renal infection which exerted a regulatory effect on normally respon-

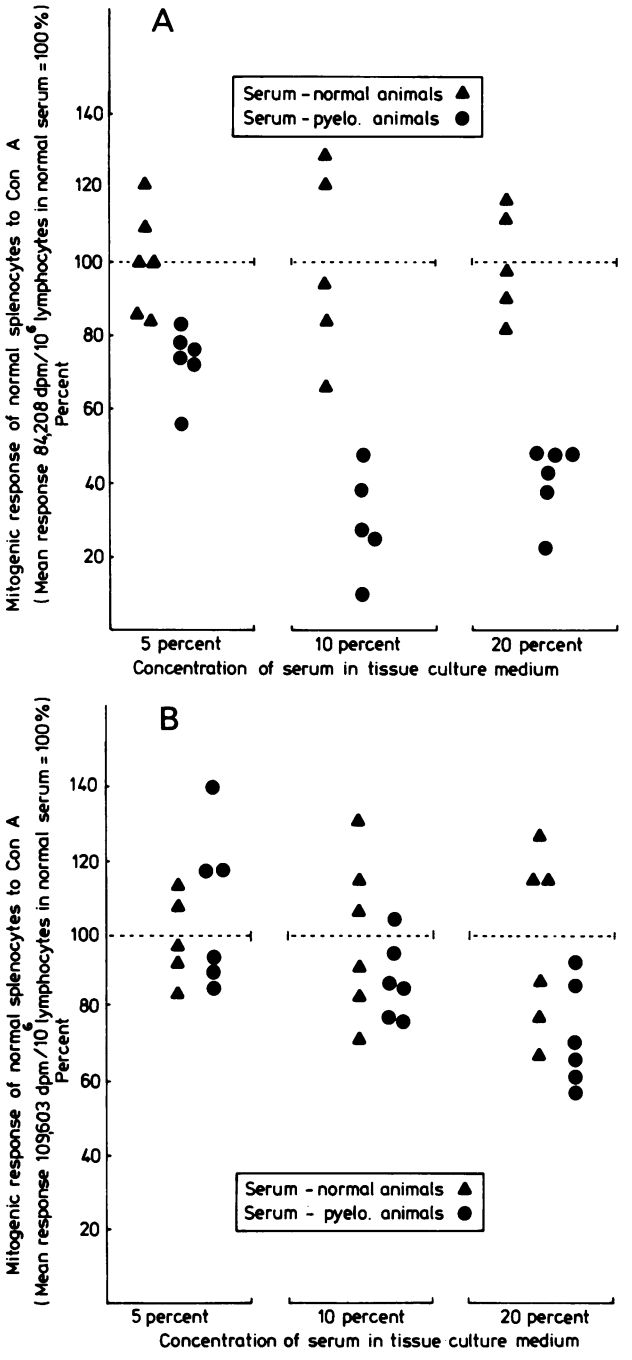

FIGURE 5 Immunosuppressive serum factors elaborated in pyelonephritis. Splenic lymphocytes from normal animals were cultured in "insert" medium containing increasing concentrations of serum (5–20%) obtained from normal animals and pyelonephritic animals challenged 72 h previously. A., con A added 24 h after the initiation of the culture; B., Con A added at the commencement of the culture. Each point from both normal and pyelonephritic animals has been expressed as a percentage of the mean response of the normal animals in each group. The mean mitogenic responses of the normal animals in groups 5A and 5B were 84,208 and 109,603 dpm/10^6 lymphocytes, respectively.

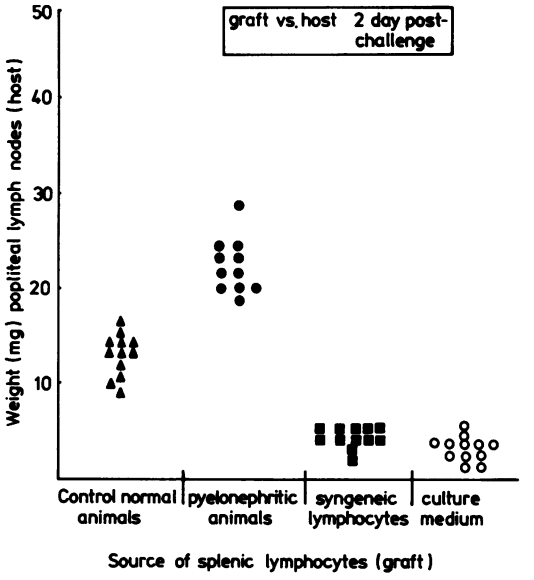

FIGURE 6 Graft vs. host response. 5×10^6 splenic lymphocytes obtained from pyelonephritic and control animals (AS2) 2 days after the establishment of renal infection, were injected into the footpads of 8-wk-old F1 recipients (AS2 × HS). Popliteal lymph node weights were determined 7 days later.

sive lymphocytes, by suppressing the T-lymphocyte response to less than 5% of its normal response.

Suppression of the T-lymphocyte response to Con A in vitro was seen as early as 2 h after the induction of renal infection but maximal suppression was found 24–72 h after infection at a time when rapid bacterial replication was taking place in the kidney. A population of suppressor cells in the splenic lymphocyte population accounted for the depressed immune response of the lymphocytes from the pyelonephritic animals and removal of these cells restored mitogenic responsiveness to the remaining splenic lymphocytes. In co-culture experiments, addition of suppressor cells to a culture of splenic lymphocytes suppressed the normal response of the splenic lymphocytes to Con A. Quantitative analysis in vivo of cell-mediated immune mechanisms was also analysis in vivo of cell-mediated immune mechanisms was also undertaken using the

TABLE II
Specific Depression of the Mitogenic Response during Renal Infection

Control group	Muscle challenge	Renal challenge
112,970*	106,369	9,126
(5,801)‡	(6,322)	(3,526)

* dpm/10^6 lymphocytes. Mean of 12 cultures of splenic lymphocytes from three animals.
‡ SEM is shown in parentheses.

host's response to a challenge of lymphocytes bearing Ag-B disparate transplantation antigens (host vs. graft). Pyelonephritic animals showed a significantly depressed response to this strong antigenic challenge and confirmed the results of the in vitro analyses of T-lymphocyte function. An immuno-suppressive factor was also found in the serum of pyelonephritic animals and this finding was confirmed in experiments in which lymphocytes from pyelonephritic animals, examined in isolation (graft vs. host reaction), were shown to have an enhanced activity.

There is increasing evidence supporting our suggestion that the depressed cell mediated immune response disclosed in these experiments is of biological significance in the pathogenesis of renal infection. (*a*) The depression of cell-mediated immunity found in the pyelonephritic animals coincided temporarily with the phase of rapid bacterial replication observed when a small inoculum of *E. coli* was introduced into the renal parenchyma. (*b*) The diminished ability of the pyelonephritic host to mount a response against a strong challenge, e.g., disparity at a major histocompatibility locus, is good evidence for a biologically significant reduction in cell-mediated immunity. (*c*) In recent experiments Ramshaw et al. (19) were able to show that administration of cyclophosphamide blocked the activity of a suppressor cell population and that this led to the restoration of the cell-mediated immune response. On the basis of their experiments we have treated pyelonephritic animals with cyclophosphamide in the anticipation that this would modify suppressor cell activity and restore host immune function. This occurred and a highly significant reduction of bacterial numbers in the kidney was found when pyelonephritic animals treated with cyclophosphamide were compared with untreated controls.[2] (*d*) The fact that the mitogenic responsiveness of splenic lymphocytes from pyelonephritic animals could be restored after the removal of the suppressor cell population and normally responsive lymphocytes failed to respond to mitogenic stimuli when co-cultured in the presence of suppressor cells. (*e*) Previously reported studies that have shown that T lymphocytes forming the lymphocytic infiltrate in the pyelonephritic kidney and obtained from the peri-renal node draining the pyelonephritic kidney show greatly reduced mitogenic responsiveness (10, 20). Finally the fact that the reduction in mitogenic responsiveness was seen during renal infection, but not after a local infection in muscle which further supports a role for the present findings in the biology of renal infection.

[2]Miller, T. E., and S. M. Phillips. The effect of cyclophosphamide on the immunobiology of renal infection. Manuscript in preparation.

We are currently investigating the nature of the putative suppressor cell which may resemble the regulatory cell described by Folch and Waksman (21). In their initial report Folch and Waksman (22) described a population of spleen cells capable of inhibiting DNA synthesis by mitogen-stimulated lymphocytes. Splenic lymphocyte suspensions from which glass-adherent cells were removed showed a marked elevation in mitogenic responsiveness. Subsequently, Folch and Waksman (23) and Bash et al. (24) with T-lymphocyte-depleted animals and selected cytotoxic agents were able to identify the suppressor cell as a T lymphocyte with surface properties defined by adhesiveness to glass. More recent experiments which have demonstrated the appearance of these suppressor cells as early as 3–6 h after antigenic challenge (18) are closely related to our own results where a depressed response of splenic lymphocytes to mitogenic challenge could be demonstrated 2 h after the induction of renal infection.

The possibility of serum factors which block or inhibit the cell-mediated immune response was also investigated and it was demonstrated that serum from animals with acute pyelonephritis was capable of causing a significant depression of the mitogenic response of T lymphocytes from normal animals. Modification of lymphocyte function occurred only when serum from pyelonephritic animals had been in contact with lymphocytes in culture for 24 h before adding mitogen. Other studies have shown the production of suppressor substances in a variety of pathological conditions including children with recurrent infections (25), immunologically mediated diseases such as graft vs. host reactions (26), and in tumor bearing mice (27, 28). The nature of the suppresive material produced as a result of these challenges has not been identified and the present studies have not differentiated between the possibility that the serum factor is a nonspecific inhibitor or cytotoxic material rather than an immunoregulatory molecule.

Functional testing of the lymphocytes from pyelonephritic animals that were nonresponsive to mitogens in vitro was carried out in vivo with the graft vs. host reaction. This showed that the functional activity of lymphocytes tested in vivo without homologous serum was enhanced. The results of this experiment strongly support data from the experiments carried out by Brooks et al. (29) which showed that lymphoid cells taken from mice made nonresponsive to H-2 incompatible skin allografts were still active in graft vs. host reactions when the lymphocytes were removed from their natural milieu. Our experiments are consistent with the hypothesis that suppressor factors in the serum of pyelonephritic animals contribute to the depressed immune status of these animals and that

this restraint is removed once the lymphocytes have been washed and transferred as an isolated suspension to allogenic recipients.

The concept that cells with immune suppressor activity may be concerned in the biology of the immune suppressor activity may be concerned in the biology of the immune response to an infectious disease has not been widely appreciated but one interesting clinical study has shown that a suppressor T-cell population may lead to hyporeactivity of potentially responsive lymphocytes in patients with fungal infections (30). In the present experiments we have provided clear evidence for a population of suppressor cells and a serum suppressive factor, both of which are specifically generated during renal infection and which modulate the host's immune response.

As the cell-mediated immune response provides a major link between the immune and inflammatory systems, a suppressed response at such a critical time may be a major factor contributing to the susceptibility of the kidney to infection and may explain the inability of host defense mechanisms to cope with an infectious challenge to the kidney.

ACKNOWLEDGMENTS

The authors wish to acknowledge the assistance of Mr. Ray Hall in the breeding and maintenance of the animals used in these experiments.

This study was supported by the Medical Research Council of New Zealand.

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