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Effect of leukocyte stimulation on rabbit immune complex glomerulonephritis

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Effect of leukocyte stimulation on rabbit immune complex glomerulonephritis. Phytohemagglutinin (PHA), a leukocyte mitogen, induces a lymphocyte and blast cell glomerulonephritis in rat renal allografts (*Cell Immunol* 13:146, 1974). The aim of this study was to assess whether PHA similarly enhances rabbit monocyte-dependent experimental, acute immune complex glomerulonephritis, and whether this effect is associated with local release of interleukin-1 (IL-1) and tumor necrosis factor (TNF). Rabbits with experimental acute serum sickness (AcSS: Group I) had focal proliferative and exudative glomerulonephritis with immune deposits, scattered subepithelial electron-dense deposits (humps), mild and transient proteinuria, normal creatinine clearance and slightly increased production of IL-1 and TNF from isolated glomeruli. Rabbits with AcSS and injected with PHA (Group II) developed severe lymphocyte and blast cell glomerulonephritis with diffuse endothelial damage; immune deposits were significantly reduced, focal subepithelial electron-dense deposits were absent, proteinuria was increased, creatinine clearance was decreased and production of IL-1 and TNF was markedly augmented as compared to rabbits in Group I. Rabbits with AcSS and injected with IL-1β and TNFα (Group V) had lesions comparable to those seen in Group II. These results show that PHA, IL-1 and TNF enhance the severity of acute immune complex glomerulonephritis, presumably by activating glomerular endothelial and mesangial cells and resident or infiltrated leukocytes.

Development of localized infections is occasionally associated with enhancement of immunologically-mediated injury elsewhere in the body.

Infections may provoke relapses or exacerbation of glomerulonephritis [1–4] and allograft rejection [5]. A similar enhancing effect was observed when the plant lectin phytohemagglutinin (PHA)—a leukocyte mitogen [6]—was injected into rats with renal allografts. A lymphocyte and blast cell glomerulonephritis, characterized by interaction of activated lymphocytes with the activated endothelium, developed in the grafts but not in the native kidneys [7]. Subsequent studies have convincingly shown that cell-mediated hypersensitivity may exert a role in the pathogenesis of experimental and human glomerulonephritis [8, 9]. The aim of the present study was to assess whether PHA similarly enhances the severity of experimental acute immune complex glomerulonephritis in the rabbit, and whether this effect is associated with a local release of the polypeptide mediators interleukin-1 (IL-1) and tumor necrosis factor (TNF).

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Methods

Animals

New Zealand White rabbits of both sexes weighing from 2 to 2.5 kg were used.

Induction of acute serum sickness

Fifty-six rabbits received a single intravenous (i.v.) injection of 250 mg/kg body weight of bovine serum albumin (crystallized BSA, Behringwerke A.F., Marburg, FRG) associated with 10 μg of *Escherichia coli* lipopolysaccharide (LPS) (Sigma Chemical Company, St. Louis, Missouri, USA) [10].

To enhance the incidence of acute serum sickness (AcSS), 1 mg/kg of body weight of BSA in complete Freund's adjuvant was administered subcutaneously three days before the intravenous injection of BSA [11]. The study included only rabbits that developed AcSS (41 rabbits, 73% of injected rabbits) as judged by glomerular immune deposit, detected by immunofluorescence microscopy technique, and demonstration of circulating anti-BSA antibodies after antigen elimination [11]. Free antibodies were determined by immunoprecipitation [12].

Twenty-four rabbits injected only with complete Freund's adjuvant and three days later with saline containing 10 μg of *Escherichia coli* LPS were used as controls.

Protocol of the experiments

After injection of BSA, rabbits were randomly assigned to five groups. Group I included 11 rabbits with AcSS, without additional treatment. Group II consisted of 10 rabbits with AcSS and injected i.v. with PHA (Wellcome Diagnostics, Hartford, UK) in a single dose of 0.5 mg/kg, seven days after the i.v. injection of BSA. Group III included 10 rabbits with AcSS, injected with human recombinant tumor necrosis factor-α (TNFα) (from Dr. Tatsuro Nishihara, Suntory Institute for Biomedical Research, Osaka, Japan; specific activity, 5 × 10^7 U/mg protein). The rabbits received a single i.v. injection of 1 μg/kg TNFα seven days after the i.v. injection of BSA. Group IV was composed of five rabbits with AcSS, injected with human recombinant interleukin-1β (IL-1β) (Istituto Sclavo,

Siena, Italy; specific activity 5.8×10^6 U/mg protein). The rabbits received a single i.v. injection of 1 μg/kg IL-1β seven days after the i.v. injection of BSA. Group V included five rabbits with AcSS, injected i.v. with TNFα and IL-1β given in single doses of 1 μg/kg TNFα and 1 μg/kg IL-1β, seven days after the i.v. injection of BSA.

Five groups of rabbits were used as controls. All these animals were injected with complete Freund's adjuvants, and three days later received a single i.v. injection of 1 ml of isotonic saline and were divided as follows: five rabbits received saline only (Group VI); seven days after the injection of isotonic saline 10 rabbits were injected i.v. with a single dose of 0.5 mg/kg PHA (Group VII); five rabbits were injected i.v. with a single dose of 1 μg/kg TNFα (Group VIII); four rabbits were injected i.v. with a single dose of 1 μg/kg IL-1β (Group IX); and five rabbits were injected i.v. with single doses of 1 μg/kg TNFα and IL-1β (Group X).

Protein excretion was measured before the i.v. injection of BSA (day 0) and at days 6, 8, 10, 12, 15. In rabbits of Groups I and II protein excretion was repeatedly measured until the time of sacrifice (day 30).

Creatinine clearance was measured in rabbits of Groups I and II at day 0 and at days 10, 15 and 30. Serum complement was measured in rabbits of Groups I and II at day 0 and at days 7 and 10 as CH50 activity [13]. In rabbits of Groups I to VI, fragments of renal tissue were obtained by surgical biopsy performed under anesthesia at days 8, 10, 12, 15, and in four rabbits of Groups I and II at day 30. In rabbits of Groups VII to X fragments of renal tissue were obtained at days 0, 1, 2 and at sacrifice, three days after the injections of cytokines. Five rabbits in Groups I and II were nephrectomized at day 10, and glomeruli were isolated to measure the production of TNF and IL-1.

Histological, immunohistological and cytochemical studies

Fragments of renal tissue obtained by biopsy or at sacrifice were processed as follows: part of tissue was fixed in 10% buffered formalin and embedded in paraffin. Sections cut at 3 μm and stained with hematoxylin and eosin or with periodic acid-Schiff (PAS) reagent were studied by light microscopy. Other sections, obtained from frozen tissue cut with a cryostat, were stained for non-specific esterase activity [14, 15]. The severity and extent of glomerular lesions were blindly and independently evaluated by two investigators selecting four parameters: hypercellularity; infiltration of polymorphonuclear leukocytes (PMN); infiltration of cells positive for non-specific esterase; fibrin thrombi and fibrinoid necrosis. In addition, the number of tubular casts was considered. Glomerular hypercellularity, fibrin thrombi, fibrinoid necrosis and tubular casts were evaluated by a semi-quantitative method of renal histology using a grading scale from 0, normal, to 5, maximum lesions [16]. The number of PMN and monocyte/macrophages was assessed by counting the total numbers of PMN and non-specific esterase-positive cells in 10 randomly-selected glomeruli divided by the number of glomeruli. A second part of the tissue was snap-frozen in liquid nitrogen and processed for immunofluorescence microscopy. Fluorescein-conjugated antibodies to rabbit IgG and C3, and to BSA were purchased from Cappel Laboratories (Malvern, Pennsylvania, USA). Staining of sections and appropriate controls were performed as previously described [17]. The amount and extent of fluorescence were assessed in a minimum of six glomeruli and was graded on a scale from 0 to 3. The intensity of immune deposits in figure 4 was expressed as average sum of scores given to all sections of renal biopsies from days 8 to 15 stained for rabbit IgG, C3, and BSA. A third part of tissue was fixed in a mixture of paraformaldehyde and glutaraldehyde [18], post-fixed in osmium tetroxide and embedded in Epon 812. Thin sections, stained with uranyl acetate and lead citrate were examined with a JEOL 100S electron microscope.

Measurement of proteinuria and creatinine clearance

Twenty-four hour urines were collected in metabolic cages. The protein concentration was measured by biuret method [19]. The creatinine clearance was calculated by measuring creatinine concentration in urine and plasma by the alkaline picrate method [20].

Production of TNF and IL-1 from isolated glomeruli

After kidney perfusion with 100 ml of sterile isotonic saline, glomeruli were isolated by sieving using successively 60, 150, 200 μm mesh stainless steel screens as previously described [21]. Glomerular preparations assessed by light microscopy exhibited less than 5% of tubular contamination. Glomeruli were counted, resuspended (10,000 glomeruli/ml) in RPMI 1640 supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin (Gibco, Glasgow, UK), and 10% fetal calf serum (Gibco), and incubated with or without 1 μg/ml LPS for 24 hours at 37°C in 95% O_2 and 5% CO_2 atmosphere. All reagents used contained less than 0.2 pg endotoxin/ml by the Limulus assay (Sigma Chemical Co., St Louis, Missouri, USA). At the end of the incubation period, the supernatants were centrifuged at 100 g and the glomeruli were removed in order to measure the proteins by Lowry method [22]. The amount of TNF and IL-1 was measured directly in the supernatants and after chromatographic purification on a Sephacryl S200 HR column (Pharmacia, Uppsala, Sweden). One milliliter of 20-fold concentrated medium was applied to 1.6% cm Sephacryl S200 HR calibrated with the following molecular weight markers (Biorad, Richmond, California, USA): ferritin (molecular wt 480,000); BSA (molecular wt 67,000); chymotrypsin (molecular wt 25,000); cytochrome C (molecular wt 12,000); and inulin (molecular wt 5,700). The column was equilibrated at 4°C with 0.1 M NaCl, 50 mM Tris-HCl buffer, pH 7.4 and samples were eluted at a flow rate of 12 ml/hr. Fractions of 2 ml were collected, sterilized by filtration and assayed for IL-1 and TNF.

TNF and IL-1 were measured in the supernatant of glomeruli isolated from kidneys of five rabbits of Group I, five rabbits of Group II, and five rabbits of Group VI 10 days after the i.v. injection of BSA or saline. Ten thousand glomeruli/ml (1.14 ± 0.4 mg proteins) were cultured for 24 hours with or without 1 μg/ml LPS and assayed. The biological activity obtained in the TNF or in IL-1 assay was abolished after absorption with specific antibodies. The antiserum to TNFα was from Cetus Corporation (Emeryville, California, USA). The antiserum to IL-1 was from Dr. C.A. Dinarello, Tufts University (Boston, Massachusetts, USA). The specificity of these two antisera was determined as previously described [23]. The biologically-active fractions of TNF were eluted from Sephacryl S-200 with an apparent molecular weight between 85 and 110 kd (corre-

sponding to polymeric TNF under non-denaturating conditions). IL-1-like activity had an apparent molecular weight ranging between 12 and 19 kd, with a peak corresponding to 14 kd.

IL-1 assay

IL-1-like activity was determined by its capacity to synergize thymocyte proliferation induced by PHA [24]. Briefly, macrophage depleted thymocytes obtained from neonatal C3H/HEJ and C3H/HEN mice were plated in flat-bottomed microtiter plates (500,000 cells/well) in complete medium with or without 0.5 μg/ml PHA and twofold serial dilutions of supernatants to be tested. After 48 hours of culture, the cells were pulsed with 1 μCi ^{3}H-thymidine (2 mCi/mmol, Amersham). After 24 hours of labelling, the medium was aspirated and 100 μl of trypsin-EDTA solution were added to each well. After an additional incubation at 37°C for 10 minutes, the cells were harvested into glass fiber filters and the ^{3}H-thymidine incorporation was measured by liquid scintillation counting. Activity was expressed as increments of mean counts of ^{3}H-thymidine uptake by cells from which the corresponding background (controls with PHA only) was subtracted. Thymocyte proliferation in the presence of RPMI with or without LPS was used as control, and showed less than 10% variation from the mean in thymidine incorporation. As a reference, human recombinant IL-1β was used. Samples positive for IL-1β-like activity were also tested after absorption with specific rabbit antibodies to human recombinant IL-1β.

TNF assay

TNF was measured by a sensitive biological assay based on the cytotoxic activity of the cytokine in the presence of an inhibitor of protein synthesis [25]. Twofold serial dilutions of samples were added together with 0.1 mg/ml of cycloheximide to cultures of human SK-MEL-109 melanoma cells sensitive to the cytotoxic activity of TNF in concentrations as low as 20 pg/ml. These cells were grown as monolayers in 24-well cluster plates, incubated with appropriate dilutions of samples, and after 20 hours washed with PBS before staining with crystal violet which was eluted and measured as described [26]. A calibration curve was constructed with human recombinant TNF to convert the cytotoxic activity of biological samples into pg/ml of TNF. Furthermore, these samples were assayed on a TNF-resistant cell line (designated R4) selected from SK-MEL-109 cells as recently described for HeLa cells [27]. The lack of cytotoxicity for TNF-resistant R4 cells in such control experiments showed that the cell death, observed with sensitive SK-MEL-109 cells, was specifically caused by TNF. In addition, TNF-dependent cytotoxicity was blocked by a rabbit polyclonal anti-human recombinant TNFα antibody. The cytotoxicity assays were carried out in triplicate and gave a standard error of < 5 percent.

Statistical analysis

The mean and standard deviation (± 1 SD) of all parameters studied by light and fluorescence microscopy was calculated for all groups of rabbits.

For statistical evaluation, data from Groups I, II, IV, V and VI were entered in a database of an IBM computer, and analyzed for statistical significance using BMDP3D one sample and two sample t-tests (BMDP, Statistical Software, Inc, Los Angeles, California, USA). P values < 0.01 were considered significant.

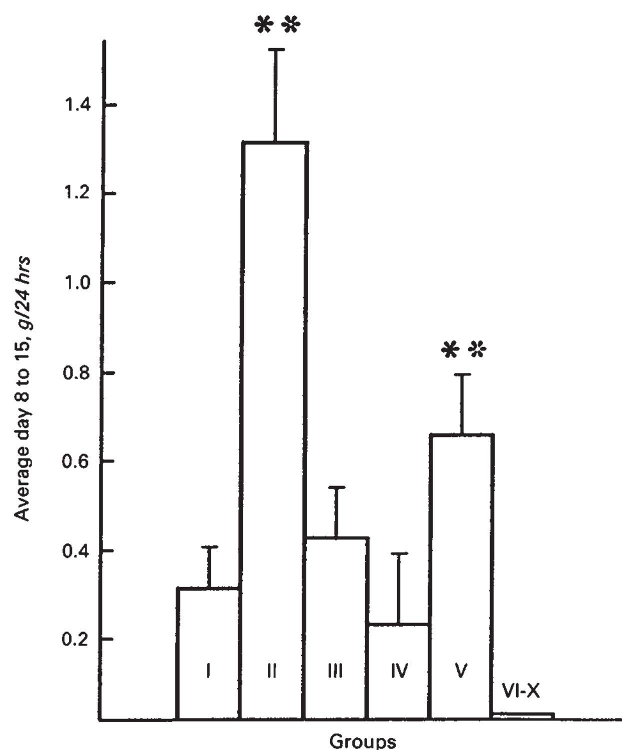


Fig. 1. *Urinary protein excretion from day 8 to day 15 are expressed as mean (± 1 SD, vertical bars) of values detected in all rabbits within each study group.* Rabbits with AcSS and injected with PHA (Group II) or with TNFα and IL-1β (Group V) had more proteinuria than rabbits with AcSS (Group I). The Student's t-test was evaluated between: Group I versus Group II, III, IV and V respectively (**$P < 0.001$).

Results

Rabbits with AcSS (Group I)

Rabbits in Group I had modest, transient, (from day 10 to day 12) proteinuria (Fig. 1), and minimal reduction of the creatinine clearance (the creatinine clearance of rabbits injected with saline, Group VI, was 3.8 ml/min/kg; Fig. 2). Granular deposits of BSA and rabbit IgG (Fig. 3) were first detectable along the glomerular basement membranes at day 8 and disappeared at day 10 and day 15, respectively. Deposits of rabbit C3 were detected at day 10, and were still present at time of sacrifice (Fig. 4). Focal and segmental proliferative and exudative lesions were present in 40 to 50 percent of glomeruli, and reached a peak at day 10. Maximum accumulation of PMN and of mononuclear cells positive for non-specific esterase was observed at day 8 and day 10, respectively (Fig. 5). Scattered subepithelial deposits of foreign material (humps) were seen by electron microscopy. Casts of proteinaceous material and cell remnants were present in the lumens of some tubules. CH50 activity of serum obtained at day 10 (7.5 ± 2.0 U/ml) was significantly reduced ($P < 0.01$) in respect to basal values (day 0 23.1 ± 5.8 U/ml) and to CH50 activity detected before immune clearance (day 7 22.4 ± 4.0 U/ml). Production of TNF

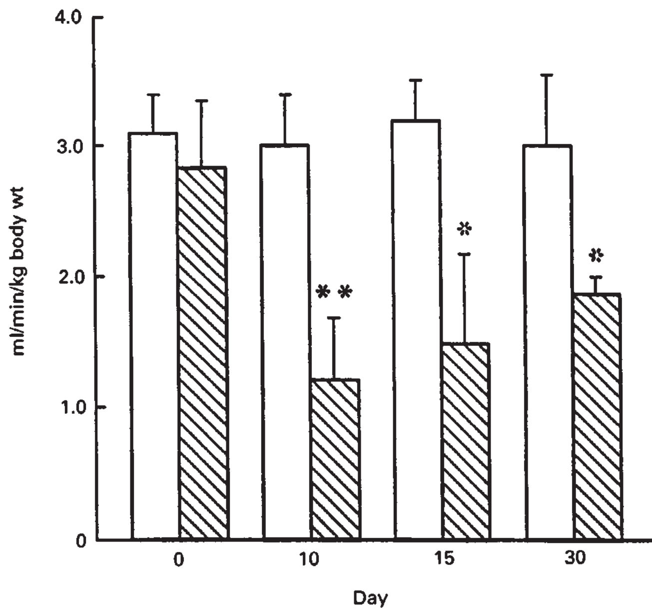


Fig. 2. *Creatinine clearance (± 1 SD) in rabbits with AcSS (□, Group I) and in rabbits with AcSS and injected with PHA (▧, Group II).* The mean creatinine clearance of rabbits injected with saline (Group VI) was 3.8 ml/min/kg. Statistical significance was assessed between rabbits of Group I and Group II (*$P < 0.01$; **$P < 0.001$). A decreased creatinine clearance persisted in rabbits of Group II 30 days after the beginning of the experiment.

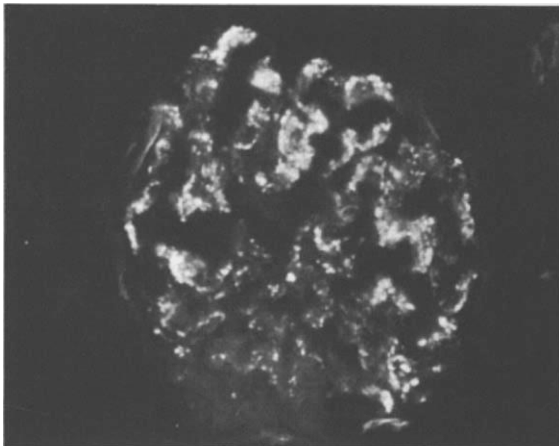


Fig. 3. *Coarse granular deposits of rabbit IgG in the glomerular capillary walls of a rabbit with AcSS without additional treatment (Group I).* × 600

and IL-1 by isolated glomeruli unstimulated or stimulated with LPS was only slightly increased as compared to rabbit injected with isotonic saline (Group VI, Fig. 6).

Rabbits with AcSS and injected with PHA (Group II)

These rabbits had persistent proteinuria (Fig. 1, $P < 0.001$) when compared to Group I and impairment of the creatinine clearance ($P < 0.01$) until the end of the observation period (Fig. 2). Glomerular immune deposits were significantly reduced and detectable at day 10 in form of fine fluorescent

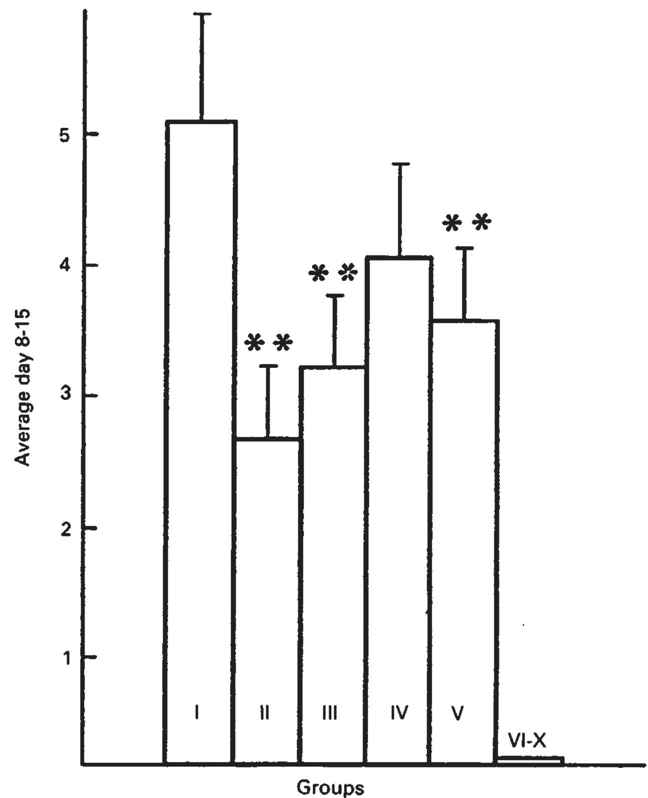


Fig. 4. *Glomerular immune deposits evaluated by immunofluorescence technique from day 8 to day 15 are expressed as mean values (± 1 SD) of the sum of scores given to sections stained for rabbit IgG, C3 and for BSA.* Rabbits with AcSS and injected with PHA (Group II) had less and different immune deposits than rabbits with AcSS (Group I). Statistical analysis was performed between Group I and Groups II, III, IV and V, respectively (** $P < 0.001$).

granules between and within inflammatory cells ($P < 0.001$, Figs. 4 and 7). About 80 percent of glomeruli had lesions that were more severe than in glomeruli of rabbits of Group I (Fig. 5, $P < 0.001$), though the severity of these lesions varied from glomerulus to glomerulus and from rabbit to rabbit. The most striking feature was the infiltration of mononuclear cells ($P < 0.001$) and, to a lesser degree, of PMN in the lumens of the capillaries, (Figs. 5, 8 and 9) and between the endothelium and the peripheral basement membrane (Fig. 12). Some of these cells resembled blast cells or activated large lymphocytes (Figs. 9, 10, 13).

Endothelial and mesangial cells were swollen and contained an increased number of ribosomes. The lumens of the capillaries were obliterated by infiltrated cells and swollen glomerular cells, and the glomerular tufts had lobular aspects (Figs. 9, 11). Early obliteration of glomerular capillaries by inflammatory cells, and increased phagocytosis, and/or a different state of immune complex aggregation were probably responsible for the decreased amount of immune deposits in the peripheral glomerular capillary walls, as compared to rabbits in Group I, and for the absence of subepithelial humps as seen by electron microscopy. Thrombi of fibrin (Fig. 10), fibrinoid necrosis (Fig. 11) and tubular casts were more frequent than in rabbits of Group I ($P < 0.01$). Reduction in CH50 activity (9.0 ± 3.1 U/ml) in serum

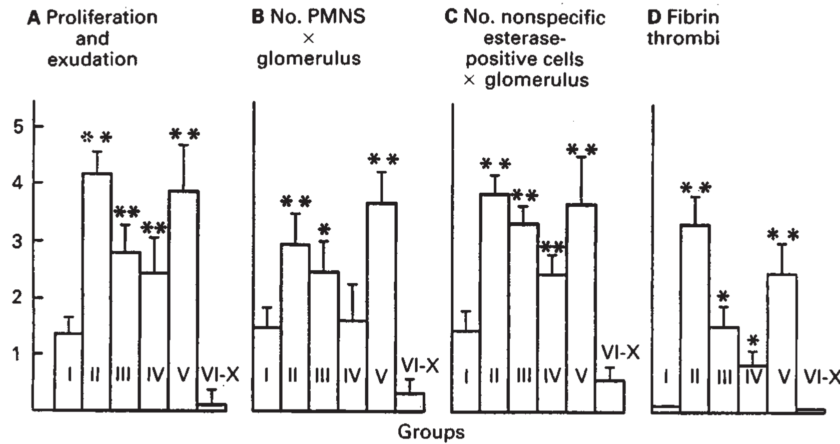


Fig. 5. *Semiquantitative evaluation of glomerular lesions.* Rabbits with AcSS and injected with PHA (Group II) or with IL-1β and TNFα (Group V) had more severe cellular infiltration and fibrin thrombi than rabbits with AcSS (Group I). The Student's *t*-test was evaluated between Group I and Group II, III, IV and V, respectively (*$P < 0.01$; **$P < 0.001$).

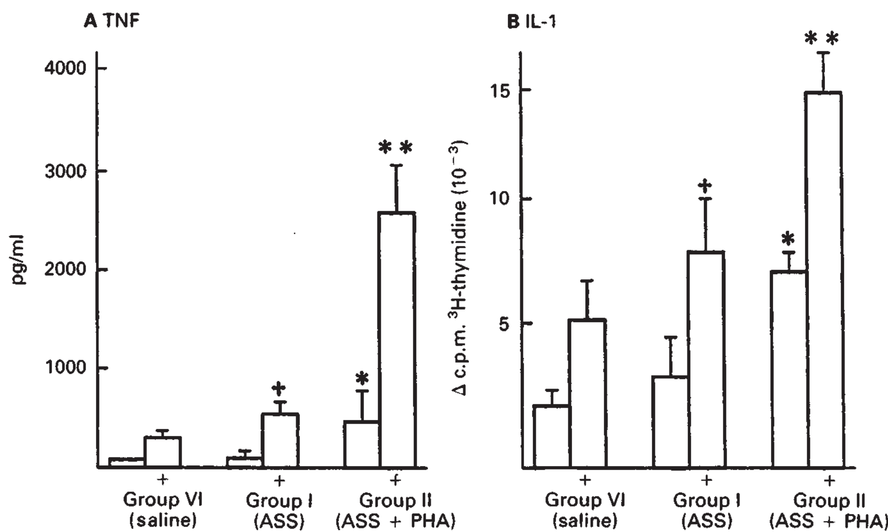


Fig. 6. *In each group of rabbits the left column indicates results obtained from unstimulated glomeruli, and the right column results obtained from glomeruli stimulated with LPS.* Stimulated glomeruli from kidneys of rabbits with AcSS and injected with PHA (Group II) produced more TNF than glomeruli from rabbits with AcSS (Group I), or normal rabbits (Group VI). Unstimulated and stimulated glomeruli from kidneys of rabbits in Group II produced more IL-1 than glomeruli from rabbits in Groups I and IV. The Student's *t*-test was evaluated between: Group VI versus Group I (+ $P < 0.01$); and Group I versus Group II (*$P < 0.01$; **$P < 0.001$).

obtained at day 10 was comparable to that of Group I rabbits. The amounts of TNF and IL-1 produced by unstimulated glomeruli were greater than in rabbits of Group I. After LPS stimulation the production of these two cytokines increased threefold (Fig. 6).

Rabbits with AcSS and injected with TNFα (Group III)

Proteinuria was slightly more elevated than in Group I (P = NS), but considerably inferior than in Group II ($P < 0.001$) (Fig. 1). The results obtained by immunofluorescence technique were similar to those obtained in Group II (Fig. 4). Glomerular cellular infiltration was increased as compared to Group I ($P < 0.001$) but less diffuse than in Group II (Fig. 5). The signs of endothelial cell activation and of interaction between endothelial cells and mononuclear cells were indistinguishable from those seen in rabbits of Group II. Intracapillary thrombi of fibrin, fibrinoid necrosis and tubular casts were seen less frequently than in Group II (Fig. 5)

Rabbits with AcSS and injected with IL-1β (Group IV)

Proteinuria was minimal (Fig. 1). The amount of immune deposits was greater than that seen in rabbits of Groups II and III, but inferior to that present in Group I (Fig. 4). As in rabbits

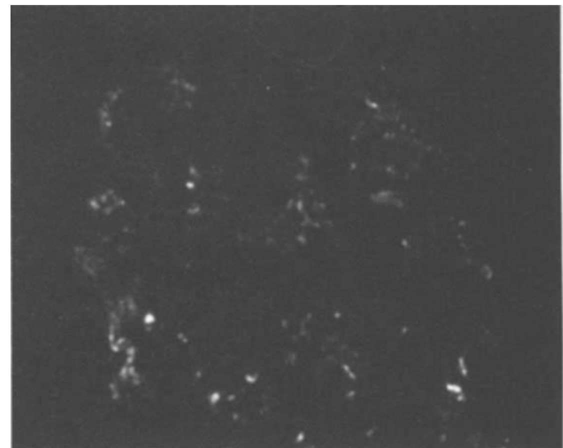
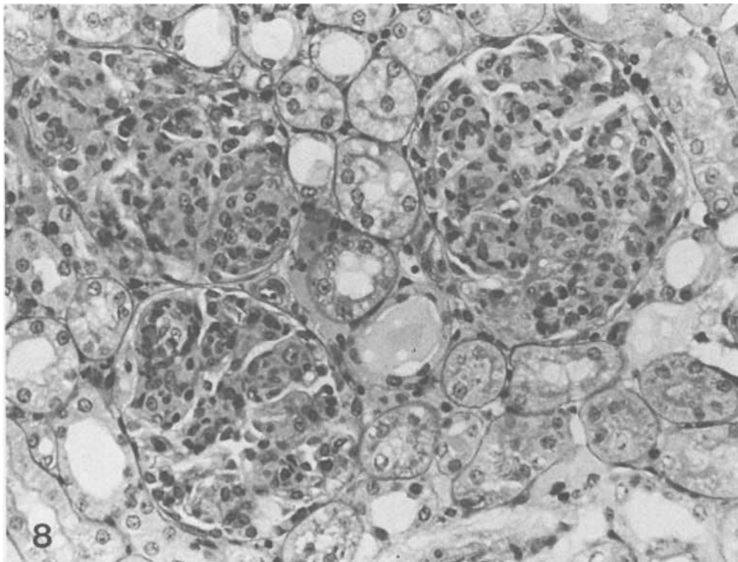


Fig. 7. *Reduced amount and fine granular pattern of rabbit IgG (compare with Fig. 3) in a glomerulus of a rabbit with AcSS and injected with PHA (Group II).* × 600

of Group III these immune deposits were small and intercellular. The degree of glomerular infiltration of mononuclear cells and PMN was more severe than in Group I and less severe than in Group III (Fig. 5). The type of lesion was similar.



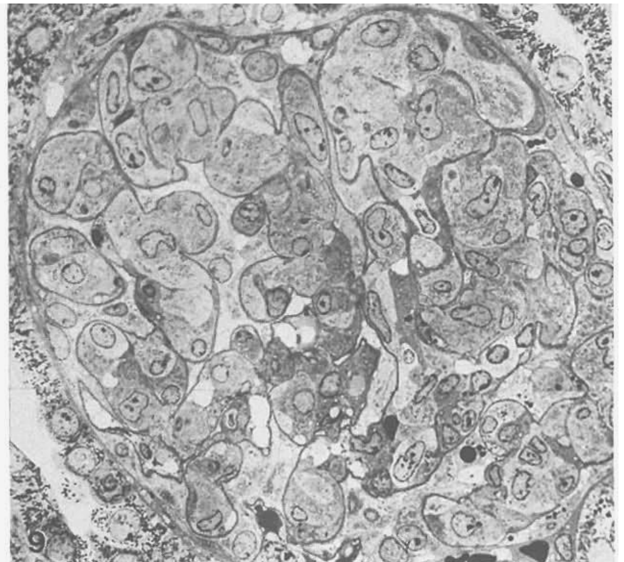
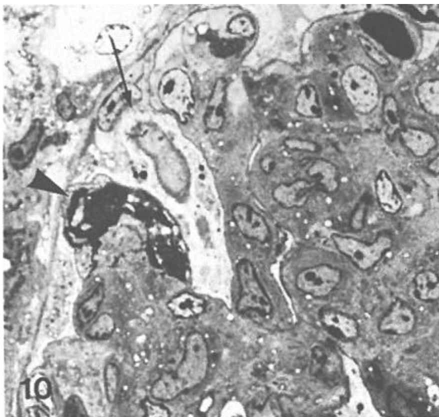


Fig. 8. *Light micrograph showing diffuse proliferative-exudative glomerulonephritis in a rabbit of Group II.* × 450

Fig. 9. *Light micrograph illustrating the massive infiltration of mononuclear cells, probably, activated lymphocytes, in the glomerular capillaries of a rabbit with AcSS and injected with PHA (Group II).* × 1,000



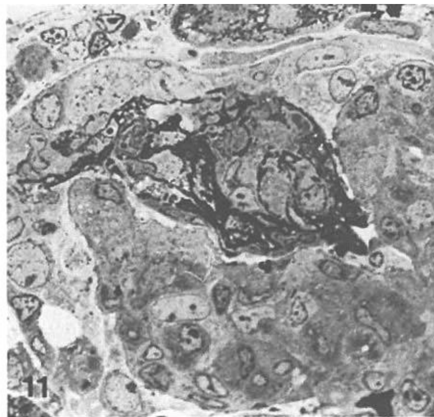


Fig. 10. *Light micrograph showing part of a glomerulus in a rabbit with AcSS and injected with PHA.* The arrowhead indicates a thrombus of fibrin, and the arrow a pale large granular mononuclear cell, probably a lymphocyte. × 1,200

Fig. 11. *Light micrograph.* Fibrinoid necrosis in a glomerulus of a rabbit with AcSS and injected with PHA. × 1,200

Rabbits with AcSS and injected with TNF-α and IL-1β (Group V)

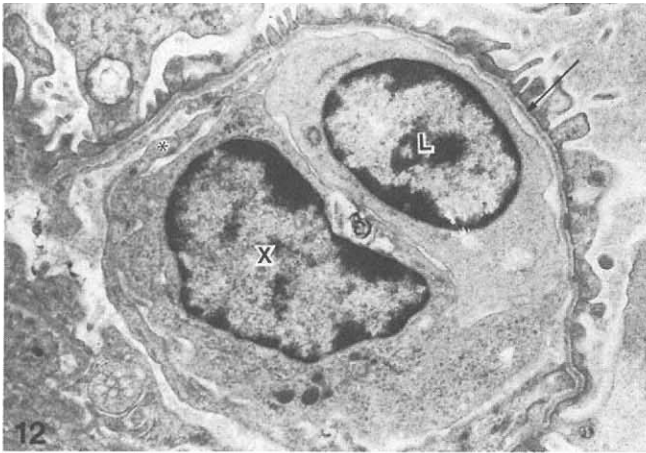
Proteinuria was increased as compared to rabbits in Group I ($P < 0.001$), and was also superior to that measured in rabbits of Groups III and IV (Fig. 1). As in rabbits of Groups II and III, the amount of immune deposits was inferior to that of Group I (Fig. 4). The glomerular lesions and the number of tubular casts were more severe and more numerous than those observed in Group I ($P < 0.001$), and similar to those seen in rabbits with AcSS and injected with PHA (Group II, Fig. 5).

Rabbits in Groups VI to X

The urinary protein excretion was normal. When examined by morphological and immunohistochemical techniques, the kidneys of these rabbits were consistently normal. Unstimulated glomeruli obtained from rabbits injected with complete Freund's adjuvants and isotonic saline (Group VI) did not produce TNF, and produced 2.5×10^{-3} cpm of IL-1 only. After stimulation with LPS glomerular production of TNF and IL-1 was increased onefold (Fig. 6).

Discussion

Administration of PHA shortly before the immune clearance of immune complexes increased the severity of morphologic and functional changes in glomeruli of rabbits with experimental AcSS. Two observations suggest that after injection of PHA, IL-1 and TNF contribute to amplify the nephritogenic response. First, lesions comparable to those induced by PHA occurred in rabbits with AcSS and injected with IL-1β and TNFα. Second, glomeruli isolated from kidneys of rabbits with AcSS and injected with PHA produced more IL-1 and TNF than glomeruli of rabbits with AcSS and not injected with the mitogen. PHA can, directly or indirectly, stimulate production of IL-1, interleukin-2 (IL-2) and TNF. IL-1 was first identified as a product of



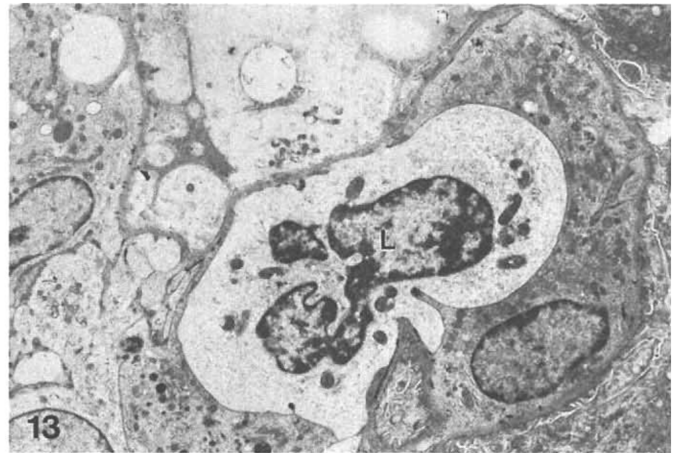


Fig. 12. *Electron micrograph showing a glomerular capillary in a rabbit with AcSS and injected with PHA.* A lymphocyte (L) has displaced the endothelium and is in contact with the glomerular basement membrane (arrow). X indicates a cell that could be a swollen endothelial cell or part of a macrophage. The asterisk indicates remnant of the endothelium. × 18,000

Fig. 13. *Electron micrograph showing a glomerular capillary from a rabbit with AcSS and injected with PHA.* L indicates a large lymphocyte. × 20,000

PHA-stimulated mononuclear cells [28]. Likewise, IL-2 was originally isolated from conditioned media of PHA-stimulated lymphocytes [29]. The stimulatory effect of PHA requires the interaction of PHA with both monocytes and lymphocytes and the production of IL-1 and IL-2 [30]. TNF may result from direct stimulation of monocyte because the mitogenic effect of PHA is not restricted to lymphocytes [6]. Moreover, certain cytokines are known to induce elaboration of other cytokines [31]. IL-2 and mitogens, including PHA, stimulate the synthesis of TNF [32] and interferon-α [33] which, in turn, promotes expression of more TNF receptors [31].

The results of our experiments are concordant with other observations suggesting that stimulation of synthesis and release, or direct administration, of proinflammatory cytokines enhance the severity of glomerular lesions. LPS augmented glomerular changes in rats injected with nephrotoxic serum [34]. Small doses of IL-1 and TNF enhanced the glomerular lesions of rats with nephrotoxic glomerulonephritis [35], and of mice with spontaneous lupus-like glomerulonephritis [36]. In these experiments, as in our observations, the most pronounced enhancing effect was obtained when IL-1 and TNF were given together [35], confirming that these two cytokines have synergistic effects [37]. One explanation why PHA induced the highest degree of enhancement is that this mitogen would provoke synthesis of both IL-1, TNF and of other cytokines, so that we examined the result of a stimulation exerted by multiple polypeptide mediators. In agreement with this hypothesis is the recent finding that IL-1β and TNFα increase mesangial cells transcription of messenger RNA for interleukin-8 and monocyte chemotactic protein-1, and the synthesis and release of interleukin-6 [38], a lymphokine that in IL-6 transgenic mice [39] and in man [40] is associated with development of proliferative glomerulonephritis.

Injection of PHA, IL-1 and TNF may exacerbate AcSS glomerulonephritis by direct stimulation of glomerular cells and resident [41] or infiltrated macrophages, or through a systemic effect. Both IL-1 and TNF activate the vascular endothelium in vitro [42] and in vivo [43], including the glomerular endothelium [44]. Endothelial activation consists of a number of responses characterized by cell hypertrophy, increased expression of class I and II histocompatibility antigens [42, 45–47], increase of the adhesiveness of neutrophils, expression of pro-coagulant molecules while, at the same time, the synthesis of plasminogen activator is decreased [42]. These changes may explain the enhancement of the inflammatory lesions, including the formation of fibrin thrombi and fibrinoid necrosis in rabbits with AcSS and injected with PHA, IL-1β and TNFα. IL-1, which can be synthesized by activated mesangial cells [48], can also stimulate them in a fashion similar to growth hormones [49] and promote synthesis of TNF [50]. On the other hand, TNF induces alteration of the glomerular endothelium, enhances intraglomerular coagulation [51] and stimulate endothelial cells [52] and macrophages [53] to produce IL-1. AcSS glomerulonephritis is monocyte/macrophage-dependent [54]. IL-1 could stimulate monocyte resident [41] or infiltrated in glomeruli. Besides, T-lymphocytes activated by IL-1 secrete IFNα that, in turn, induces macrophages to express class II antigens and other indicators of activated state [42]. Thus, systemic injection of PHA, IL-1β and TNFα could enhance the production of proinflammatory peptides in glomeruli, in addition to any direct effect that they may have on glomerular cells.

Glomerular infiltration of large lymphocytes morphologically similar to natural killer (NK) cells or IL-2-activated lymphocytes [55] is seldom observed in experimental and human glomerulonephritis. This peculiar cellular infiltration may be the consequence of the stimulus of cytokines on NK cells and CD8+ lymphocyte [56, 57], which could contribute to damage glomerular endothelial and mesangial cells with their cytolytic pore-forming protein [58].

In conclusion, the plant lectin mitogen PHA enhances the severity of glomerular morphologic and functional changes in rabbit with AcSS, increasing glomerular production of IL-1 and

TNF. Lectins are carbohydrate-binding proteins present in pathogenic bacteria, viruses, and alimentary tract antigens [59] that could be associated with relapses or exacerbations of glomerulonephritis. The results described are relevant to understanding how systemic infections, or infections at distal sites of the body, exacerbate cell-mediated glomerular lesions [1, 6] by a reaction broadly akin to the acute phase response [60].

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