Antilymphocyte Serum In Nephrotoxic Serum Nephritis[1] (34685)

EDWIN R. FISHER AND BERNARD FISHER

Departments of Pathology and Surgery, University of Pittsburgh and Veterans Administration Hospital, Pittsburgh, Pennsylvania 15240

The precise pathogenetic mechanism involved in the development of nephritis in rats following the injection of serum from rabbits immunized with homogenates of rat kidney (NTS) is uncertain. However, there is considerable evidence to suggest that the immediate, acute reaction designated as the homologous phase of nephrotoxic serum nephritis (NTN) results from the binding of antibody to antigen represented by glomerular basement membrane. Progression to chronic nephritis, not unlike that observed in humans, has been considered to result from either a reaction between host's antibodies formed in lieu of the heterologous globulin fixed in the kidney (1) or damaged renal tissue induced by the initial injury (2).

Since administration of antilymphocyte serum (ALS) or globulin has been observed to interfere with a variety of immune reactions, it was considered worthwhile to examine its effect on NTN. Information derived from such a study might provide some insight into certain aspects of its pathogenesis.

Materials and Methods. One ml of NTS was intravenously injected into 30 adult female Sprague-Dawley rats weighing 150-200 g. Sixteen of these received daily intraperitoneal injections of 2 ml of ALS for 3 days prior to injection of NTS and daily thereafter for 28 days. Fourteen rats received a similar course of normal goat serum (NGS) prior to and after NTS injection.

NTS was prepared in rabbits by intraperitoneal injections of homogenates of kidneys from Sprague-Dawley rats as described previously (3). ALS was raised in goats by injecting pooled Sprague-Dawley thymocytes and nodal lymphocytes as described previously (4). ALS utilized exhibited a leukoagglutination titer greater than 1:64.

All animals were housed in individual metabolism cages and received a standard ration and water *ad libitum*. Daily urinary protein was measured as described previously (3). Total leukocyte and lymphocyte counts were performed at weekly intervals in all animals.

Groups of rats were sacrificed by aortic puncture at 1 and 7 weeks following injection of NTS. BUN, serum cholesterol, total lipid and protein were estimated as described previously. Portions of kidney, spleen, thymus, adrenal, lung, and lymph nodes were fixed in Zenker's acetic fluid. Aliquots of kidney were also immediately frozen on Dry Ice or fixed in 1% osmium tetroxide buffered with Veronal to pH 7.4. Zenker's fixed tissue was dehydrated, imbedded in paraffin, and stained with hematoxylin and eosin and the periodic acid–Schiff technic. Frozen tissue was sectioned in a cryostat at —20° and stained with sheep antirabbit gamma globulin (anti-RGG), rabbit antirat gamma globulin (anti-rGG), rabbit antiguinea pig complement (anti-C) and rabbit antigoat gamma globulin (anti-GGG) conjugated with fluorescein isothiocyanate and examined by fluorescence microscopy. Osmium-fixed tissue was imbedded in Maraglas, and ultrathin sections were examined with a Philips EM 300 electron microscope.

Results. Onset and degree of daily urinary protein (Fig. 1), gain in body weight, serum BUN, cholesterol, total lipid and protein were comparable in ALS- and NGS-treated rats sacrificed at 1 and 4 weeks (Table I). Proteinuria was immediate and sustained at moderate levels up to 12 days at which time it became massive. Peritoneal cavities were moist in all NTS-treated rats.

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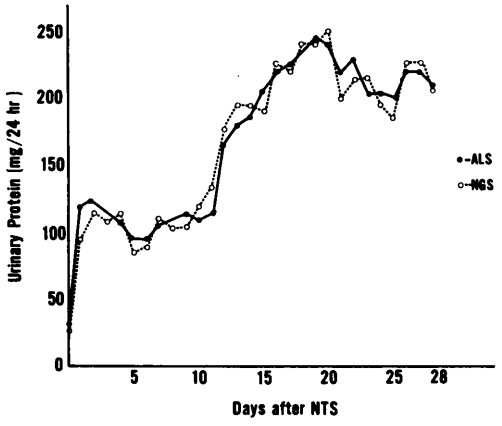

FIG. 1. Daily urinary protein of rats with NTN, treated with ALS and NGS.

Total leucocyte and lymphocyte counts were depressed in ALS-treated animals only (Fig. 2).

Histological examination of kidneys revealed thickening and splitting of glomerular basement membranes as well as mild proliferative change in all rats sacrificed at 1 week regardless of treatment. Kidneys from rats sacrificed at 4 weeks exhibited similar alterations and, in addition, apparent thickening of the mesangium.

No difference in histological appearance of lymph nodes or thymus was recognized in ALS- and NGS-treated rats. No difference in ultrastructural appearance of glomeruli from ALS- and NGS-treated rats was observed. Glomerular epithelial cell changes consisting of cytoplasmic swelling, vacuolization, and loss of foot processes were conspicuous and, at 4 weeks, increase in basement membrane-like mesangial material was present. No changes in the lamina densa were observed.

Kidneys from both groups of rats revealed linear localization of RGG (NTS) and C at 1 and 4 weeks. rGG also appeared similar in both. Although its distribution did not appear significantly different than has been observed in rats not treated with NTS, its intensity was slightly greater than in the latter. No localization of GGG (ALS) was noted within the kidney.

Discussion. Results indicate that ALS failed to alter the clinical, biochemical, morphological including ultrastructural and immunohistochemical characteristics of the homologous and so-called autologous phases of NTN. Since ALS has been observed to suppress initial recognition of antigen including rabbit serum protein (5) by antibody cells (5–7), these findings tend to minimize the role of host antibody to rabbit gamma globulin or damaged renal substance in the pathogenesis of the so-called autologous phase of NTN. It is of interest in this regard that ALS has been noted to inhibit the development of auto-allergic encephalitis (5). Although host's gamma globulin which could represent such antibody was detected in glomeruli of all animals with NTN as noted by others (1), its similarity in distribution to that noted in normal untreated controls suggests that it may represent deposition of circulating native protein. In this light, the

TABLE I. Body Weight and Biochemical Changes in NTN.

Treatment (weeks)	No. of rats	Change in body wt (g)	(mg/100 ml) BUN[a]	Cholesterol	Total lipid	Total protein[a] (g/100 ml)
ALS						
1	8	+24	25	162 ± 22[b]	220 ± 30	5.1
4	8	+72	28	184 ± 15	274 ± 35	4.6
NGS[c]						
1	7	+30	29	174 ± 18	240 ± 28	4.9
4	7	+67	27	170 ± 20	287 ± 32	5.0
Normal	10		25	80 ± 22	151 ± 26	6.6

[a] Pooled samples.
[b] Standard deviation.
[c] NGS = normal goat serum.

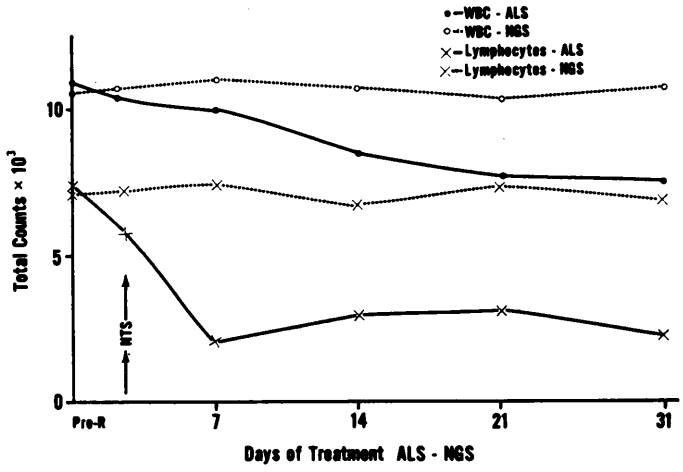

FIG. 2. Leukocyte and lymphocyte counts of rats with NTN, treated with ALS and NGS.

more intense reaction noted in rats with NTN might be interpreted as a reflection of greater concentration of this protein in such diseased kidneys. On the other hand, Hammer and Dixon (8) have noted that rats made tolerant to rabbit gamma globulin at birth failed to develop a chronic (autologous) form of NTN. Further, possible complexes of host antibody, complement, and antigen were noted only in nontolerant rats receiving NTS by electron microscopy (9). The failure to detect renal localization of ALS (goat gamma globulin) by immunofluorescence, as well as the similarity of histologic and ultrastructural appearance of the lesions in these experiments to those observed in rats receiving NTS, only minimizes the significance of possible antigen–antibody complexes to this heterologous protein in the pathogenesis of the lesions encountered, or, alternatively, host formation of antibody to goat globulin was minimal or absent.

The failure to observe alteration in the parameters of NTN investigated is analogous to our previous experience concerning the effect of neonatal thymectomy on this form of experimental renal disease (10). This is consonant with evidence indicating that ALS interferes with the central production of antibodies which is related to lymphoid elements dependent upon integrity of the neonatal thymus (11). The failure to observe histologic changes in thymus or paracortical portions of lymph nodes does not appear paradoxical. Lymphocytic hyperplasia (12), depletion (5), as well as no change (13) in such tissues has been observed following ALS administration. That the ALS utilized in these experiments was active is evident by its *in vivo* lymphopenic and *in vitro* agglutination effects. However, it is appreciated that neither may be essential for its immunosuppressive activity noted in other situations.

Summary. ALS administration failed to influence the clinical, biochemical, morphological including ultrastructural and immunohistochemical features of the so-called homolous, and autologous phases of nephrotoxic serum nephritis in rats. This indicates that progression of this form of experimental renal disease is not necessarily dependent upon immunologic functions abrogated by ALS.

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