RNA Induced Anti-Kidney Auto-Antibodies

Experimental immunological renal diseases may be induced in several species of animals by immunization with a variety of homologous[1-4] or heterologous kidney antigens[5-7]. In addition it has been demonstrated that an experimental nephritis may be induced in normal lambs by serum from nephritic sheep previously immunized with homologous and heterologous renal antigens[8,9].

In previous papers the presence of a RNA carrier of the antibody template in blood serum and lymphoid tissues of immunized rabbits was reported[10-13]: such RNA is able to elicit in non-immunized animals a precocious antibody response to the same antigens used for immunizing the animals source of RNA.

On the basis of these results the following investigations were undertaken to ascertain whether it was possible to induce autoimmune anti-kidney antibody response in normal rats with RNA from serum and lymphnodes of rabbits immunized against rat renal antigens.

Reddish rabbits of QR strain from Nossan S.p.A. (Milan), weighing 2.5–3 kg, were immunized by s.c., i.m. and in foot-pads injections of rat kidney homogenates containing soluble and insoluble renal cortex antigens incorporated in complete Freund's adjuvant (1:1 v/v): each antigen dose consisted of 5 mg protein nitrogen; the whole immunization cycle consisted of 4 injections made at weekly intervals; 2 weeks after, the animals received a last booster injection and they were then exsanguinated 15 days later. Circulating antibody titres were determined before each injection and 4 days after the last one by the usual complement fixation technique (50% hemolysis). At the end of immunization cycle the average antibody titre was 1:2560 (1:1280–1:10,240).

From the pooled immune rabbit sera and, as a control, from a pool of normal rabbit sera, RNA was extracted as previously described[11]. In addition RNA was extracted from popliteal lymphnodes of both foot-pads immunized and normal rabbits according to a technique similar to that described elsewhere[15].

Young Wistar rats weighing about 150 g were carefully selected by several urinary controls to exclude proteinuria, hematuria and any pathological change in their urine and urinary sediment composition; immediately before starting the treatment, also the BUN values were estimated according to the urease method[16]; only animals giving absolutely normal results were used and grouped into 4 lots of 12 animals each.

The animals of each lot were injected i.v. with a single 0.3 mg/100 g body wt. dose of RNA extracted respectively from (1) normal rabbit serum, (2) normal rabbit lymphnode, (3) immunized rabbit serum and (4) immunized rabbit lymphnode. After this treatment each animal was subjected to urinary controls at 12 h intervals over a period of 48 h: 90% of rats treated with RNA from immunized rabbits showed a mild proteinuria starting from the 12th h and progressively increasing during 48 h; at this time the average urinary protein lost was 1.76 g/1000 ml and it was always accompanied by severe hematuria. Urinary sediment analysis showed many leukocytes, hematies, hyaline and granular casts. At the end of the 48 h interval, a blood sample for the BUN and specific anti-kidney circulating antibodies determination was withdrawn: the BUN values for all the animals treated with RNA from immunized rabbits were significantly increased and, on average, about 100% higher in respect to the basal values; specific circulating antibodies against rat renal antigens were detectable with the complement fixation technique. Each urina and blood control on the animals treated with RNA from non-immunized rabbits did not show any pathological change (Table).

The animals were then killed (except for 3 for each lot which were kept under continuous observation for the next 30 days) and both kidneys quickly removed: 1 kidney was weighed and appropriately fixed sections (Bouin) were saved for histological and histochemical studies with

[1] R. W. Steblay, Fedn. Proc. *24*, 693 (1965).
[2] W. Heymann, D. B. Hackel, S. Harwood, S. G. Wilson and J. L. P. Hunter, Proc. Soc. exp. Biol. Med. *100*, 660 (1959).
[3] E. V. Hess, C. T. Ashworth and M. Ziff, Ann. N.Y. Acad. Sci. *124*, 323 (1965).
[4] E. R. Unanue, F. J. Dixon and J. Feldmann, J. exp. Med. *125*, 163 (1967).
[5] R. W. Steblay, J. exp. med. *116*, 253 (1962).
[6] W. Heymann, E. P. Kmetec, S. G. Wilson, J. L. P. Hunter, D. B. Hackel and F. Cuppage, in *Immunopathology Third International Symposium* (Ed. P. Grabar and P. Miescher, (Schwabe Co. Publishers, Basel 1963), p. 240.
[7] E. R. Unanue and F. J. Dixon, J. exp. med. *125*, 149 (1967).
[8] R. A. Lerner and F. J. Dixon, J. exp. Med. *124*, 431 (1966).
[9] V. Rudofsky and R. W. Steblay, Fedn. Proc. *25*, 569 (1966).
[10] L. Michelazzi, A. Novelli, G. Nanni and I. Baldini, Experientia *20*, 703 (1964).
[11] L. Michelazzi, I. Baldini, A. Novelli and G. Nanni, Nature *205*, 194 (1965).
[12] L. Michelazzi, G. Nanni, I. Baldini and A. Novelli, Nature *207*, 1406 (1965).
[13] L. Michelazzi, A. Novelli, I. Baldini, U. M. Marinari and G. Nanni, Experientia *21*, 585 (1965).
[14] L. Michelazzi, A. Novelli, I. Baldini, U. M. Marinari and G. Nanni, Sperimentale *115*, 276 (1965).
[15] L. Michelazzi, I. Baldini and U. M. Marinari, Experientia *24*, 387 (1968).
[16] A. L. Chaney and E. P. Marbach, Clin. Chem. *8*, 131 (1962).

Urine and blood changes in rats 48 h after treatment with RNA from various sources

RNA from	Proteinuria g/1000 ml	Hematuria	Casts	BUN g/1000 ml	Specific antibody titre	No. of determinations
Serum of normal rabbits	–	–	–	0.25 ± 0.07	< 1:10	12
Popliteal lymphnodes of normal rabbits				0.22 ± 0.05	< 1:10	12
Serum of rabbits immunized with rat renal antigens	1.76 ± 0.65	+	+	0.54 ± 0.16	1:320 (1:80–1:1280)	12
Popliteal lymphnodes of rabbits immunized with rat renal antigens	1.45 ± 0.48	+ +	+ – +	0.49 ± 0.12	1:640 (1:160–1:1280)	12

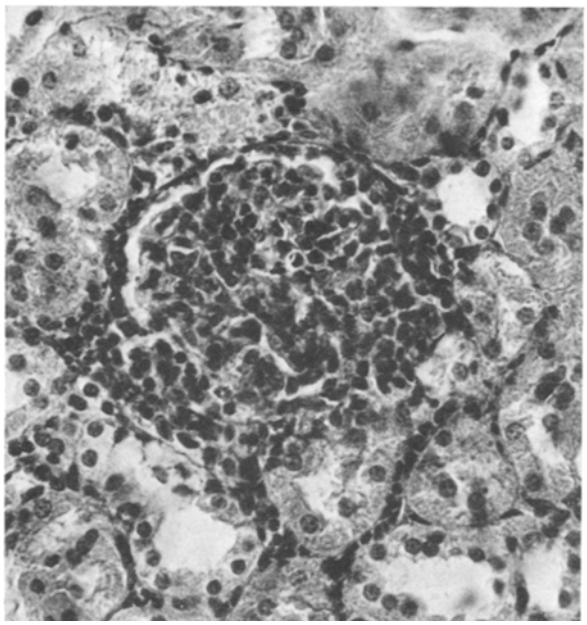

Fig. 1. Glomerulus of a rat treated with RNA from serum of rabbit immunized with rat renal antigens (Hematoxylin-Eosin, × 400).

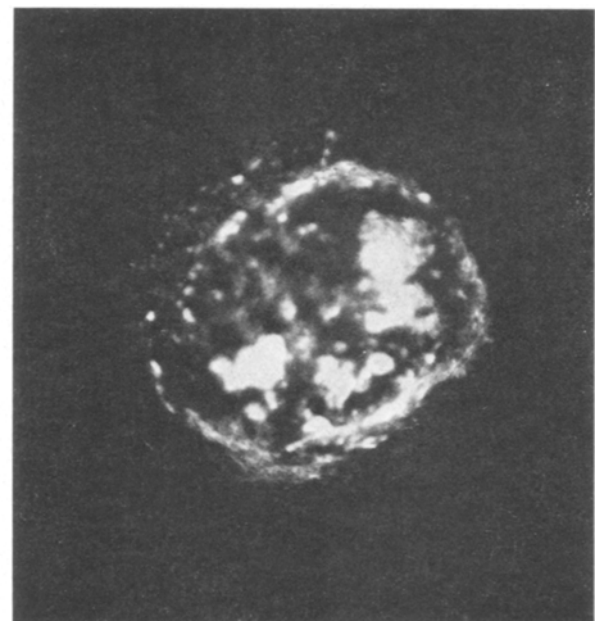

Fig. 2. Antigen-antibody complexes revealed by immunofluorescence (anti-complement fluorescent serum) in a glomerulus of a rat treated with RNA from serum of rabbit immunized with rat renal antigens (× 400).

hematoxylin-eosin stain and with the methods of NOVELLI[17], VAN GIESON and MACMANUS; with the second kidney, after a careful perfusion with saline to remove any residue of blood, rolled films were prepared according to the technique of DIXON[18] which were incubated with anti-rat globulins fluorescent serum for the usual indirect immunofluorescence test[19]; moreover, some of the slides were treated with anti-complement fluorescent serum according to a technique used by KLEIN et al.[20] to confirm the presence of antigen-antibody complexes.

The morphological investigations put in evidence precocious glomerular injury which developed simultaneously with the occurrence of the proteinuria and consisted of basal membrane thickening, swelling and abnormal cellularity of glomerular tufts with the presence of PAS positive material in the Bowman's spaces and infiltration of mono and polymorphonuclear leukocytes; also the convoluted tubules showed mild degenerative features going from cloudy swelling to the necrosis of several cells lining the proximal convoluted tubules with the presence in the lumina of jalinous and PAS positive material. Several glomeruli showed also adhesions between the capillary tuft and Bowman's capsule (Figure 1).

The lesions described were particularly evident in the animals injected with serum RNA, attained the maximum of severity 48 h after challenge and remained in the acute phase till at least the 10th day; after this time the histopathological features became progressively similar to those of subacute and chronic nephritis.

Immunological studies of rolled films by the immunofluorescence methods showed the presence of fluorescent material casts into the glomerular loops, in the Bowman's spaces and, to a less extent, in the tubular epithelium. The technique using anti-complement fluorescein labelled serum appeared to be more sensible and specific because stained glomerular casts with a scarce background fluorescence (Figure 2); the anti-rat globulins fluorescein labelled serum was less specific for glomerular antigen-antibody complexes which appeared more diffusely fluorescent in a sensibly fluorescent background, probably due to the presence of some residues of rat globulins.

Controls made with the lots of animals treated with RNA extracted from serum and lymphnodes of normal rabbits did not show any clinical or histological change.

Many aspects of these morphological alterations concerning both histological[4,21] and immunofluorescence[4,20,21,22] findings are closely similar to those observed in the course of autoimmune nephritis obtained in several experimental conditions.

All these clinical and morphological observations, and mainly the fact that fluorescein labelled anti-complement globulins as well as fluorescent anti-rat globulins can be fixed on to the glomerular lesions, suggests that they include antigen-antibody complexes. This means that RNA extracted from animals immunized with rat renal antigens is able to induce, in untreated rats, synthesis of anti-kidney auto-antibodies.

Riassunto. Sono stati descritti alcuni aspetti funzionali, morfologici ed istoimmunochimici di una nefropatia sperimentalmente indotta nel ratto mediante l'uso di un RNA estratto da siero e da tessuto linfoide di conigli immunizzati con antigeni renali di ratto.

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[17] A. NOVELLI, Boll. Soc. ital. Patol. *5*, 145 (1958).
[18] K. C. DIXON, Br. J. exp. Path. *45*, 481 (1964).
[19] R. A. LERNER, R. J. GLASSOCK and F. J. DIXON, J. exp. Med. *126*, 989 (1967).
[20] P. KLEIN and P. BURKHOLDER, Dt. med. Wschr. *45*, 2001 (1959).
[21] T. S. EDGINGTON, R. J. GLASSOCK and F. J. DIXON, J. exp. Med. *127*, 555 (1968).
[22] R. J. GLASSOCK, T. S. EDGINGTON, J. I. WATSON and F. J. DIXON, J. exp. Med. *127*, 573 (1968).