Nephritogenic Antibodies in Lupus Nephritis

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VOGT, A., BATSFDORD, S. and MORIOKA, T. Nephritogenic Antibodies in Lupus Nephritis. Tohoku J. Exp. Med., 1994, 173 (1), 31-41 — A critical discussion of data on the possible role of IgG3 cryoglobulins, cross-reactive anti-DNA antibodies and anti-DNA idiotypes in the pathogenesis of lupus nephritis is included. A further possibility involving cationic nuclear autoantigens is presented in detail. Histone was employed as a model antigen, representative of this group. Histones were shown to have high affinity for the rat GBM; they could also act as planted antigen and induce IC formation; furthermore they were able to mediate the binding of highly anionic DNA. The demonstration of histones in glomerular deposits in both lupus mice and patients with SLE is important evidence, linking such molecules with the kidney lesions. —— lupus nephritis; nuclear autoantigens; charge; histones; ubiquitin

The question as to which autoantibodies are nephritogenic in SLE has been fuelled with new interest by recent findings and has led to new concepts. For a long time the pathomechanisms of lupus nephritis seemed to be clear. In analogy to the classic studies in serum sickness (Germuth 1953; Dixon et al. 1958) it seemed to be almost proven that circulating immune complexes are the causative agent in immune complex glomerulonephritis and that the glomerular deposits in lupus nephritis consisted of DNA and anti-DNA antibodies. This concept became even more convincing when Izui et al. (1976) showed that DNA possesses affinity for collagen and for the glomerular basement membrane (GBM) in vitro. However, DNA does not possess any affinity for the GBM in vivo. Intravenous and intrarenal injection of DNA fragments of various size does not produce localization in the glomeruli in higher amounts than seen with control antigens (Cukier et al. 1986; Stöckl et al. 1990). If anti-DNA antibodies play an essential role in the pathogenesis of lupus nephritis - and many findings support this - the question remains how this antibody and the corresponding antigen become deposited in the glomeruli. This also holds true for any other antigen-antibody system.

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Which facts have to be accounted for in any concept of the pathogenesis of lupus nephritis?

Lupus nephritis is a frequent organ manifestation in SLE. The granular pattern of the glomerular deposition of autologous immunoglobulin and complement points to an immune complex disease. The immune deposits are mainly located subendothelially, but can also be found subepithelially and within the mesangium (Zollinger and Mihatsch 1978). The variation in glomerular histopathology, as well as the variety of clinical symptoms in SLE point to more than one pathomechanisms in lupus nephritis. One can assume that the glomerular deposits consist of, or contain, nephritogenic antibodies or nephritogenic antigens, or both. The immunoglobulins in renal eluates obtained from SLE patients and from lupus mice consistently showed specificity for DNA (Lambert and Dixon 1968; Winfield et al. 1977). Among the candidates for nephritogenic autoantibodies in SLE, antibodies to dsDNA therefore have to be accorded first place. Theoretically glomerular deposition of antibodies may be the result of direct binding to distinct structures of the GBM or may be mediated by the corresponding antigen. In the latter case the chance of glomerular deposition of the immune complexes is higher if the antigen possesses affinity for the GBM. Studies during the last decade have taught us that cationized proteins may reveal high affinity for the GBM (Batsford et al. 1980; Oite et al. 1982). In particular, glomerular deposits located in the subepithelial region point to the presence of a cationic component in the glomerular bound immune complexes (Vogt 1984).

What promotes glomerular deposition of anti-DNA antibodies?

Among the serum autoantibodies to nuclear antigens in SLE (Table 1) the

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Autoantibody frequency (%)</th>
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<tbody>
<tr>
<td>Native DNA</td>
<td>40</td>
</tr>
<tr>
<td>Denatured DNA</td>
<td>75</td>
</tr>
<tr>
<td>Histones</td>
<td>70</td>
</tr>
<tr>
<td>Sm</td>
<td>30</td>
</tr>
<tr>
<td>Nuclear RNP</td>
<td>32</td>
</tr>
<tr>
<td>SS-A/Ro</td>
<td>35</td>
</tr>
<tr>
<td>SS-B/La</td>
<td>15</td>
</tr>
<tr>
<td>Ribosomal RNP</td>
<td>10</td>
</tr>
<tr>
<td>Ubiquitin</td>
<td>70</td>
</tr>
<tr>
<td>Hsp 90</td>
<td>50</td>
</tr>
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* Disease-specific autoantibodies and diagnostic markers
  From Tan (1989) and from Muller et al. (1988).
anti-dsDNA antibodies are highly disease-specific (Tan 1989). Antibodies of IgG class with high affinity for DNA and the capacity to activate complement are associated with renal disease (Glassock et al. 1991). The other qualities listed in Table 2 which are connected with glomerular deposition of antibodies have been derived from various experimental studies (Eilat 1985; Fournié 1988; Wilson 1991).

**IgG3 cryoglobulins**

Impressive results have been achieved with monoclonal cryoglobulin of IgG3 isotype, derived from MRL-lpr/lpr mice (Lemoine et al. 1992). Transplantation of some, but not all, hybridomas producing such monoclonal cryoglobulins into the peritoneal cavity of MRL/BALB F₁ hybrids resulted in renal alterations resembling the wire-loop lesions seen in lupus nephritis. Specificity for dsDNA was not a prerequisite for this kind of nephritogenicity. In addition, affinity for the GBM seems not to be the underlying pathomechanism. Experiments involving intravenous injection of purified cryoglobulins failed to demonstrate affinity for the GBM. Since not all transplanted hybridomas producing IgG3 cryoglobulins revealed nephritogenicity, some as yet unknown physicochemical property of the cryoglobulin is apparently important in provoking glomerular lesions. Whether cryoglobulins of IgG3 isotype play a role in the pathogenesis of lupus nephritis in man is not known, but could be easily tested. A more detailed survey on these interesting IgG3 cryoglobulins can be found in the contribution of Izui et al. in this volume.

**Crossreactive anti-DNA antibodies**

As with cryoglobulins, the nephritogenic potency of crossreacting anti-DNA antibodies (Table 3) derived from lupus mice has been tested by transplanting the relevant hybridomas into the peritoneal cavity of normal mice. Those hybridomas which 3 to 5 weeks after onset of ascites produced glomerular immune deposits in the recipients were declared to be nephritogenic. The underlying pathomechanism has not been clarified; it is not clear whether such so called nephritogenic monoclonals are deposited as circulating immune complexes or are reacting directly with crossreacting structures in the glomerulus. Intravenous

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**Table 2. What promotes glomerular deposition of anti-DNA antibodies?**

<table>
<thead>
<tr>
<th>Antibody Type</th>
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<tbody>
<tr>
<td>IgG isotype</td>
</tr>
<tr>
<td>High affinity</td>
</tr>
<tr>
<td>IgG3 cryoglobulin</td>
</tr>
<tr>
<td>Crossreactivity, polyreactivity</td>
</tr>
<tr>
<td>Peculiar idiotypes</td>
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<tr>
<td>Targeting via cationic nuclear antigens</td>
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injection of 1 to 2 mg of purified nephritogenic monoclonals are claimed to result in the same IF pattern of deposited globulin as in the mice that received the corresponding hybridomas (Vlahakos et al. 1992).

Quantitative data about direct binding of the antibodies to glomerular structures are available only for one nephritogenic crossreacting monoclonal, designated H241. The target antigen of this antibody in the glomerulus is thought to be laminin, because its reactivity was shown to be inhibited by laminin, in addition to ds and ssDNA (Sabbaga et al. 1989). Less than 0.1 percent (5–20 ng) of intravenously injected 125I labelled antibody was bound to the glomeruli of both kidneys when isolated 1 h after injection, a figure which is hardly indicative of affinity for the GBM. Altogether there seem to be differences in the capacity of crossreacting monoclonal anti-DNA antibodies to form glomerular immune complexes in the recipient normal mouse when the corresponding hybridoma is growing in the peritoneal cavity. The amount and pattern of glomerular deposition vary and the immune deposits are often found in the mesangium. The observed kidney injury, as judged by the resulting proteinuria (1–3 mg/day) is only marginal. A serious omission is that control studies with transplantation of hybridomas producing monoclonals with specificity other than for DNA have not yet been performed. The properties speculatively linked with the proposed nephritogenic capacity, as for example a peculiar antigen binding region, or the crossreactivity leading to multiple antigen-antibody interactions, do not tell us how the glomerular immune deposits are formed.

**Anti-DNA idiotypes**

The linkage of nephritogenicity to a peculiar anti-DNA antibody idotype, assumed by several groups, has recently been questioned, as the idiotypes in question are not restricted to anti-DNA antibodies but can be frequently found on anti-bacterial antibodies in infectious diseases, and even on human myeloma proteins (Table 4, Watts and Isenberg 1990). The concept that only a subset of anti-DNA antibodies with certain idiotypes are nephritogenic is based on the

<table>
<thead>
<tr>
<th>Antigens</th>
<th>References</th>
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<tbody>
<tr>
<td>Polynucleotides</td>
<td>Lafer et al. 1981</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>Shoenfeld et al. 1983</td>
</tr>
<tr>
<td>Proteoglycans</td>
<td>Faaber et al. 1986</td>
</tr>
<tr>
<td>Other nuclear antigens</td>
<td>Migliorini et al. 1987</td>
</tr>
<tr>
<td>Cell surface proteins</td>
<td>Jacob et al. 1984</td>
</tr>
<tr>
<td>Platelet membrane</td>
<td>Watts et al. 1990</td>
</tr>
<tr>
<td>Fibroblast cytoskeleton</td>
<td>Rauch et al. 1987</td>
</tr>
<tr>
<td>Bacterial antigens</td>
<td>Carroll et al. 1985</td>
</tr>
</tbody>
</table>
observation that particular anti-DNA antibody idiotypes can be detected in glomerular deposits in murine and human SLE (Isenberg and Collins 1985; Diamond and Schwartz 1987; Kalunian et al. 1989; Muryoi et al. 1990). In addition, in NZB/W F1 mice the onset of lupus nephritis can be down-regulated by administering anti-idiotypic antibody to anti-DNA (Hahn and Ebling 1984). It is even possible to induce a lupus nephritis-like disease in normal C3H.SW mice following immunization with human monoclonal anti-DNA antibody bearing the public idiotype 16/6 (Mendlovic et al. 1988), which is found in 50 percent of SLE patients. With regard to the observation that the accused idiotypes are not restricted to so called nephritogenic anti-DNA antibodies (Table 4), the most likely explanation is that an immune response to antibodies carrying a distinct idiotype causes disturbances of idiotype-anti-idiotype networks. This may be relevant for the initiating event, causing synthesis of autoantibodies, but does not provide insight into the mechanism of glomerular deposition of these antibodies, the essential prerequisite for development of renal injury.

Possible role of cationic antigens

Regarding the serum autoantibodies it is striking that these are directed against nuclear antigens of extreme opposite charge (Table 1). Nuclear antigens like DNA and RNA are strongly anionic molecules while those like histones and most of the ribonucleoproteins are highly cationic. Our own concept is based on the assumption that the deposition of antibodies found in glomerular eluates of lupus nephritis are mediated by cationic nuclear autoantigens and that the interaction of these cationic antigens with the negatively charged GBM is the pertinent initiating event in the pathogenesis of lupus nephritis. The glomerular deposition of the disease-specific anti-DNA antibodies are thus thought to be mediated by these antigens. The background to our concept was provided by experimental studies with cationized antigens, performed by ourselves and other groups over the last ten years (Batsford et al. 1980; Gallo et al. 1981, 1983; Oite et al. 1982). The pertinent findings are listed in Table 5. Cationized antigens as well as cationic immune complexes bind to the glomerular capillaries when

### Table 4. Frequencies of common DNA idiotypes in serum from SLE and infectious disease

<table>
<thead>
<tr>
<th></th>
<th>16/6 (%)</th>
<th>BEG 2 (%)</th>
<th>PR 4 (%)</th>
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<tbody>
<tr>
<td>Normal subjects</td>
<td>4</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>SLE patients</td>
<td>40</td>
<td>8</td>
<td>70</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>60</td>
<td>34</td>
<td>37</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>37</td>
<td>14</td>
<td>nd</td>
</tr>
<tr>
<td>Lyme disease</td>
<td>65</td>
<td>60</td>
<td>60</td>
</tr>
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injected intravenously, size and degree of positive charge being critical. In
general, the higher the pI and the larger the size, the higher the affinity for the
GBM (Vogt et al. 1982). Besides this, highly cationic proteins can mediate the
deposition of anionic antigens (Barnes and Venkatachalam 1984). In view of the
fact that, among the known nuclear autoantigens in SLE, there are several of
extreme positive charge, it seemed promising to regard cationic nuclear antigens as
candidates for mediation of glomerular deposition of DNA and anti-DNA anti-
bodies. As a model antigen for experimental studies we chose histones. His-
tones are closely linked to DNA, so that they are probably released together into
the circulation. Their physiological function is the packing of DNA into
chromatin, which is built up out of repeating units, the nucleosomes. The
nucleosomes are composed of a core particle, which consists of an histone octamer
(H2A, H2B, H3, H4)$_2$, surrounded by a 145-bp DNA molecule and linker DNA
($\approx$55bp) to which histone H1 is bound (Nelson et al. 1982). All histones are
extremely cationic, the pI being between 10 and 11. We started our experimental
studies with histone aggregates. Histones in monomeric form have a size of 11 to
22KD and are too small to become fixed to the GBM, for any appreciable length
of time; histones, however, do aggregate spontaneously. When 200 $\mu$g of ag-
ggregated histone subfractions were injected into the left renal artery via the aorta,
6 to 16% of the administered material was found in the isolated glomeruli after
15 min, compared to 0.2 percent of lysozyme, which was used as a control antigen
(Schmiedeke et al. 1989). Intensive deposition of histone in a capillary pattern
could be seen when cryostat sections were stained for histones. Kinetic studies
with $^{125}$I-labelled histones revealed that histones planted to the GBM persist long
enough to serve as a target for circulating antibody. About 2 percent of the
injected 200 $\mu$g of histone H2A were still found in the isolated glomeruli 8 hr later.
Subsequent intravenous injection of anti-histone antiserum from the rabbit led to
a marked deposition of IgG along the GBM. As with cationized proteins, glomer-
ular planted histones could mediate the glomerular deposition of subsequently
injected anionic DNA. The DNA was located of along the glomerular capillaries
and was absent in control experiments, where prior histone injection was omitted.
In quantitative studies where 200 $\mu$g histone H3 were injected into the left kidney
via the aorta followed by 100 $\mu$g of $^{125}$I-labeled ss- or dsDNA fragments of 500 to
7000 base pairs, 39 percent and 35 percent of the DNA probes, respectively, were

<table>
<thead>
<tr>
<th>Table 5. Résumé of major findings with cationized proteins</th>
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<tr>
<td>1. Interaction with glomerular capillary wall when pI exceeds 9.0</td>
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<tr>
<td>2. Persistence size dependent, should exceed 50 KD</td>
</tr>
<tr>
<td>3. Act as target for circulating antibody</td>
</tr>
<tr>
<td>4. Established immune complexes cannot be removed by competing polycations.</td>
</tr>
<tr>
<td>5. Mediate deposition of anionic antigens</td>
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</table>
bound to the glomeruli at 15 min. As with the planted histones, the deposited DNA was accessible for circulating antibody. That histones are involved in the pathogenesis of lupus nephritis is supported by other recent findings. In the majority of kidneys of proteinuric lupus mice (NZB/NZW F1 and GvH) we were able to demonstrate the presence of histones in the glomerular deposits (Fig. 1, Schmiedeke et al. 1992). In a multicenter study of renal biopsies, where clinicians and scientists from Japan, Venezuela, Italy and our country have participated, we were able to demonstrate histone in the glomerular deposits of 26 biopsies out of a total of 39 cases of human lupus nephritis (Stöckl et al. 1994). The specificity of the staining was confirmed by using antisera raised in rabbits to synthetic histone peptides. What mechanisms other than in situ deposition and in situ immune complex formation are conceivable or are even more likely? The first event may be the deposition of histone- or histone-DNA complexes. But histone-DNA-antibody complexes should also have affinity for the GBM when they occur in the circulation. We have started to test individual substructures of nucleosomes for their affinity for the GBM. Nucleosomes, as one could expect, have no affinity for the GBM, the string of DNA surrounding the histone core will give it a negative overall charge. Recently we were able to show that intravenously injected core histone subunits localize along the GBM and can act as target antigen for subsequently injected soluble DNA-anti-DNA complexes.

Fig. 1. Immunofluorescence micrograph of a kidney from a lupus mouse that developed graft-versus-host disease with proteinuria and ascites. Stained with rabbit serum directed against the N-terminal region (residues 1-21) of histone H3. Granular glomerular deposits are seen in the mesangial area and along the capillary walls. With permission of Clinical and Experimental Immunology (ref. 35).
This situation is quite likely to occur in vivo in the course of SLE. We are now studying whether soluble immune complexes may be prepared from histone octamer-DNA fragments and anti-DNA antibodies. Such complexes would in our opinion be the most likely candidates for initiating lupus nephritis in SLE.

A role for ubiquitin

One of the most frequent autoantibodies found in SLE sera is directed against the 7KD heat shock protein, ubiquitin (Table 1) (Muller et al. 1988). Histone H2A also occurs in a stable ubiquitinated form (U-H2A) and the branched region of U-H2A is an epitope that is also frequently recognized by SLE sera (Plaue et al. 1989). We were recently able to demonstrate positive glomerular staining by immunofluorescence to both ubiquitin and U-H2A in 10 and 21 out of 39 renal biopsies respectively from patients with SLE (Stöckl et al. 1994). This raises the possibility that histone can introduce ubiquitin to the glomerulus via a charge interaction, thus offering further epitopes for IC formation.

Possible role of cationic antibody

Regarding the importance of charge in the pathogenesis of lupus glomerulonephritis, attention has been drawn to the possible role of cationic antibodies in the formation of glomerular deposits. Compared to their serum antibody counterparts, IgG antibodies with reactivity for DNA in the renal and glomerular eluates of lupus kidney have been found to be more cationic (Ebling and Hahn 1980; Dang and Harbeck 1984), but there is also a nonconfirmatory report (Yoshida et al. 1985). Analyzing monoclonal anti-DNA antibodies derived from SNF1 and NZB mice for charge, the highest pI determined were 8.8 to 9.0 (Gavalchin et al. 1987). Whether these antibodies alone or complexed with DNA fragments will localize along the glomerular capillaries when injected intravenously is not known, but could easily be experimentally tested.

Concluding remarks

Critical appraisal of current ideas on the pathogenesis of glomerulonephritis in SLE has led us to propose a role for cationic nuclear antigens. This concept has now been brought to the stage at which stringent testing is both required and possible.

References

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39) Tan, E.M. (1989) Antinuclear antibodies: Diagnostic markers for autoimmune dis-
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