Identification of a pathogenic epitope involved in initiation of Heymann nephritis

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Contributed by Marilyn Gist Farquhar, August 24, 1992

ABSTRACT Heymann nephritis is an experimental autoimmune disease model for human membranous nephropathy. We have recently identified a pathogenic epitope, clone 14 (C14), responsible for formation and deposition of glomerular immune complexes that is contained within the small subunit of the Heymann nephritis antigenic complex (HNAC). HNAC is a heterodimer composed of a large subunit designated gp330 and a smaller (44 kDa) subunit, which is immunologically identical to the receptor-associated protein. In this study, we prepared antibodies to fusion proteins with C-terminal deletions in the C14 sequence and assessed their ability to promote formation of immune deposits (IDs). When IgG specific for the shortest truncated fusion protein (C14/Δ3; 86 amino acids) was injected into rats, small IDs developed. In contrast, when IgG raised against the full-length C14 sequence was depleted of its reactivity toward the C14/Δ3 fusion protein (C14/Δ3-fp), no IDs could be detected. These data indicate that at least one pathogenic epitope is contained within the N-terminal 86 amino acids of C14. Since the IDs induced with the C14/Δ3-fp-specific IgG are smaller than those induced with the polypeptide-specific anti-gp330 antibodies, it is likely that other epitopes in addition to those expressed by the C14/Δ3-fp are required for formation and growth of immune complexes.

Membranous nephropathy is a major cause of chronic renal failure requiring subsequent replacement of kidney function by hemodialysis or transplantation (1). The hallmark of this autoimmune disease is the formation and deposition of subepithelial immune complexes in kidney glomeruli (1). In other autoimmune disorders, specific polypeptide epitopes are directly involved in antigenicity (e.g., see ref. 2). Similarly, the initial events of Heymann nephritis (HN) are thought to occur through the binding of circulating antibodies to accessible domains within the HN antigenic complex (HNAC) exposed on the surface of the glomerular epithelial cell (3). Characterization of these pathogenic epitopes is essential for development of effective therapies. We have recently identified a cDNA [clone 14 (C14)] encoding one such epitope by screening an expression library with antibodies eluted from glomerular immune deposits (IDs) of HN rats (4). The cDNA was shown to encode a 319-amino acid polypeptide that contains a pathogenic epitope, because antibodies raised against a C14 fusion protein (C14-fp) produced passive HN in rats, and immunization of rats with C14-fp resulted in the active disease. Subsequently, we demonstrated that the C14 polypeptide, originally thought to be part of gp330, represents the smaller subunit of the gp330–44-kDa heterodimeric complex (3). In addition, we showed (3) that the 44-kDa protein is immunologically identical to the receptor-associated protein that is associated with the low density lipoprotein (LDL)

receptor-related protein/α₂-macroglobulin receptor (LRP) (5–8).

In this report, we set out to further define the nephritogenic epitope present in the 44-kDa subunit through C-terminal deletion analysis of the C14 sequence. Several truncated fp were prepared, but only antibodies specific for the smallest fp construct (C14/Δ3-fp; 86 amino acids) resulted in subepithelial deposition of immune complexes when injected into rats. In addition, immunodepletion of C14/Δ3-fp reactive antibodies eliminated the potency of the IgG preparation to promote IDs. From these data, we conclude that at least one nephritogenic epitope is presented within the N-terminal 86 amino acids of the 44-kDa subunit of HNAC.

MATERIALS AND METHODS

Deletion Clones of C14. A cDNA clone (C14) of 1260 base pairs (bp) was ligated into the *EcoRI* site of Bluescript plasmid SK(+) II (Stratagene) as described (4). Unidirectional deletions from the C terminus of the coding region of C14 were generated by using exonuclease II (9) as follows. The C14 Bluescript plasmid was linearized with *Kpn* I and *Hind*III, which digest the plasmid DNA downstream of the C14 cDNA. This DNA was treated with exonuclease III to remove mononucleotides starting from the 5' overhang generated by *Hind*III and proceeding unidirectionally into the C14 sequence. Aliquots were removed at various times, and the exposed single strands were removed by digestion with the mung bean nuclease to generate blunt ends. Plasmids containing truncated cDNAs were digested with *EcoRI* and *Bss*HI followed by a fill-in reaction with Klenow and phenol/chloroform extraction. The truncated cDNAs, C14/Δ1, C14/Δ2, and C14/Δ3, were selected and sequenced with Sequenase (United States Biochemical). C14/Δ1, C14/Δ2, and C14/Δ3 contain 654, 376, and 259 bp and encode 218, 125, and 86 amino acids (see Fig. 1), respectively. For ligation in λgt11, the DNAs were separated in a 1% agarose gel and isolated with GeneClean (Bio 101, La Jolla, CA). Phosphorylated *EcoRI* linkers (75 pmol; Stratagene) were ligated to each DNA (0.5 pmol) using T4 DNA ligase and then digested with *EcoRI*. After heating at 65°C for 15 min, the DNA was precipitated twice with ethanol in the presence of 3.5 M ammonium acetate and then ligated to dephosphorylated *EcoRI*-digested λgt11 arms at a 1:1 molar ratio. The phage DNA was packaged using Gigapack II Plus (Stratagene) and then plated on *Escherichia coli* Y1088 in the presence of isopropyl β-D-thiogalactopyranoside and 5-bromo-4-chloro-3-indolyl β-D-galactoside to select for recombinants. The phages were lysogenized into *E. coli* Y1089 for production of fp. Using this strategy, only those cDNAs

Abbreviations: C14, clone 14; fp, fusion protein(s); GBM, glomerular basement membrane; HN, Heymann nephritis; HNAC, HN antigenic complex; IDs, immune deposits; LDL, low density lipoprotein; LRP, LDL receptor-related protein/α₂-macroglobulin receptor.

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ligated in one orientation—i.e., the same sense as the intact C14-fp in λ gt11—will yield β -galactosidase-C14 fusion proteins. cDNAs in the other orientation are not expressed because of a stop codon 1 bp from the *Bss*HI site.

Antibodies. Anti-gp330 IgG was raised in a rabbit by immunization with gp330 purified on a BioGel 5m (Bio-Rad) column from detergent-solubilized rat kidney microvillar fractions (10). Specific IgG was affinity purified on a CNBr Sepharose 4B (Pharmacia) column to which isolated gp330 (≈ 1 mg/ml) was bound (4). Glomerular IgG was acid eluted from kidney cortex homogenate of rats injected 3 days previously with anti-gp330 IgG and purified on protein A-Sepharose 4B (Pharmacia) as described (10). Anti- β -galactosidase IgG was raised by immunization of a rabbit with β -galactosidase (Sigma) and affinity purified on a β -galactosidase Sepharose column. Anti-C14-fp IgG was produced in a rabbit immunized with C14-fp purified by preparative SDS/PAGE and electroeluted as described (11); the sera were affinity purified on a C14 β -galactosidase column. IgGs specific for the truncated C14-fps (C14/ Δ 1, C14/ Δ 2, and C14/ Δ 3) were prepared by affinity purification of anti-C14-fp antisera on Sepharose 4B columns to which the respective fp was immobilized. Bound IgG was eluted with 0.1 M acetic acid, pH 3.2/150 mM NaCl/0.1% gelatin, neutralized, dialyzed against phosphate-buffered saline (PBS), and concentrated with Aquacide II (Calbiochem). Anti-C14-specific IgG depleted of its reactivity with C14/ Δ 3 was prepared by repeated adsorption on a C14/ Δ 3-fp column.

Immunoblotting. *E. coli* Y1089 induced to overexpress C14-, C14/ Δ 1-, C14/ Δ 2-, and C14/ Δ 3-fp were solubilized in SDS sample buffer, separated by SDS/PAGE, and transferred onto nitrocellulose (4). Immunoblotting was performed by incubation with anti-gp330, anti-C14-fp, or anti-C14/ Δ 3-fp IgG (10–20 μ g/ml), or with IgG eluted from glomeruli of rats with passive HN (11) followed by detection with goat anti-rabbit IgG conjugated to alkaline phosphatase (Promega). Immunoblotting was also performed with anti-C14-fp IgG immunodepleted of its reactivity with C14/ Δ 3-fp. When used for immunoblotting of recombinant fusion proteins, these antibodies were first depleted of their nonspecific antibodies to *E. coli* components by adsorption to an SDS lysate of *E. coli* Y1089 bound to Sepharose 4B (4). Controls consisted of immunoblots on *E. coli* lysates containing irrelevant fp and on purified β -galactosidase.

Induction of IDs with fp-Specific IgGs. Affinity-purified IgG (40–50 μ g) specific for either gp330, C14-fp, or the truncated fp were injected into the tail vein of male Sprague-Dawley rats (250 g). Some rats were injected with ≈ 4 mg of rabbit anti-C14-fp IgG, which was depleted of its reactivity with the C14/ Δ 3-fp. Controls were injected with ≈ 1 mg of rabbit anti- β -galactosidase IgG. After 3 days, the animals were sacrificed, and their kidneys were perfused via the abdominal

FIG. 1. Truncated C14 β -galactosidase fp. A Bluescript plasmid containing the 1260-bp C14 DNA was digested with exonuclease III to generate C14 deletion clones. The deleted DNAs were subcloned into λ gt11 vector for expression as β -galactosidase fp, C14/ Δ 1, C14/ Δ 2, C14/ Δ 3, of 218, 125, and 86 amino acids (aa), respectively. β -gal, β -galactosidase.

FIG. 2. Expression of β -galactosidase fp of the C14 deletion clones. *E. coli* Y1089 induced to overexpress C14-, C14/ Δ 1-, C14/ Δ 2-, or C14/ Δ 3-fp were solubilized in SDS sample buffer, separated by SDS/PAGE, and stained with Coomassie blue. The full-length C14-fp has an apparent mass of ≈ 150 kDa, whereas those of the truncated fp show apparent masses of 138, 128, and 123 kDa, respectively (arrowheads). Lane MW, molecular size markers.

aorta with minimal essential medium. One kidney was removed unfixed and used for preparation of cryostat sections; the other was fixed by perfusion with paraformaldehyde/lysine/periodate as described (12).

Immunocytochemistry. Cryostat sections (5 μ m) prepared from unfixed kidney tissue of rats injected with fp-specific IgGs were incubated in rhodamine-conjugated goat anti-rabbit F(ab')₂ (Cappel Laboratories). Semithin cryostat sections (3 μ m) of paraformaldehyde/lysine/periodate-fixed tissue were cut on a Reichert Ultracut E equipped with a F4-E cryoattachment, quenched in 10 mM glycine in PBS, and incubated with rhodamine-conjugated goat anti-rabbit F(ab')₂

FIG. 3. Immunoblots on full-length C14-fp and truncated fp. Lysates of *E. coli* Y1089 induced to overexpress C14-, C14/ Δ 1-, C14/ Δ 2-, and C14/ Δ 3-fp were separated by SDS/PAGE, transferred to nitrocellulose, and immunoblotted with IgG raised against the entire C14-fp and affinity purified on a C14/ Δ 3-fp column (A), anti-C14-fp IgG that was depleted of its reactivity with the C14/ Δ 3-fp by exhaustive absorption to a C14/ Δ 3-fp column (B), and IgG eluted from glomeruli of a rat with passive HN induced by injection of anti-gp330 IgG 3 days before sacrifice (C). Lane MW, molecular size markers.

in PBS with 1% bovine serum albumin (BSA) for 12 hr at 4°C, washed in PBS with 1% BSA, mounted in glycerol containing paraphenylenediamine, and examined in a Leitz or Zeiss fluorescence microscope.

RESULTS

Expression of Truncated C14-fp. A 1260-bp cDNA of C14 encoding 319 amino acids (4) was used to produce unidirectional deletions to derive the truncated cDNAs C14/Δ1, C14/Δ2, and C14/Δ3, which encode 218, 125, and 86 amino acids, respectively, from the N terminus of C14 (Fig. 1). These cDNAs were cloned into λgt11 for expression as β-galactosidase fp in *E. coli* Y1089. SDS/PAGE analysis of the *E. coli* lysates demonstrated fp of 138 (C14/Δ1), 128 (C14/Δ2), and 123 (C14/Δ3) kDa (Fig. 2), which is in agreement with the lengths of their respective cDNAs.

Identification of Two Antigenic Epitopes on the C14 Polypeptide. We have previously reported that, by immunoblotting, the full-length C14-fp is specifically recognized by anti-gp330 IgG, anti-C14-fp IgG, and the IgG eluted from glomeruli of rats with passive HN (4). The reactivity demonstrated by the anti-gp330 IgG toward the 44-kDa subunit and C14-fp is most likely due to copurification of gp330 and

the 44-kDa subunit of HNAC during preparation of the antigen (3). To map the anti-C14-fp binding sites presented within the C14 polypeptide, anti-C14-fp IgG was separated into two subfractions by immunoabsorption. The fp of the smallest peptide, C14/Δ3-fp, was coupled to activated Sepharose resin and two IgG fractions were prepared: one fraction that bound specifically to the N-terminal 86 amino acids of the C14 polypeptide sequence and a second fraction that was depleted of C14/Δ3-fp reactivity. The binding sites of the two IgG subfractions on the C14 and truncated fp were then mapped by immunoblotting. As expected, the C14/Δ3-reactive IgG fraction recognized the full-length C14-fp as well as all three N-terminal truncated fp (Fig. 3A). However, the IgG fraction devoid of C14/Δ3-fp reactivity bound only the full-length C14 polypeptide sequence (Fig. 3B). These results indicate that there are two major antigenic determinants presented within the C14 sequence: one within the N-terminal 86 amino acids, and one located within the C-terminal 101 amino acids of the full-length C14-fp that are deleted from the truncated fp.

In principle, either one of these two epitopes could represent a pathogenic epitope responsible for antibody binding *in vivo* leading to accumulation of subepithelial IDs. To determine which of these IgGs is capable of binding to HNAC *in*

FIG. 4. Direct immunofluorescence of glomeruli of rats that were injected with the same amounts (120 μg) of either anti-gp330 or anti-C14-fp IgG. Sections of precisely 3 μm were cut by cryomicrotomy to permit direct visual comparison of the size and density of IDs. (A) Glomeruli from a rat injected with rabbit IgG raised against purified gp330 and affinity purified on a column containing purified gp330. Note large size and high density of IDs in peripheral capillary loops (arrowheads). (B) Glomeruli from a rat injected with the same amount of rabbit IgG raised against the purified C14-fp and affinity purified on a C14-fp column. Smaller, fewer, and more dispersed IDs are seen. There is also labeling of the mesangial (M) areas. L, capillary lumen; US, urinary space. (×900.)

vivo, we eluted the IgG fraction bound to the glomeruli of rats with passive HN induced by injection of anti-gp330 IgG and tested this antibody preparation by immunoblotting on the full-length and truncated fp. The eluted IgG recognized the full-length C14-fp and all the truncated fp. The fact that it recognized the C14/ Δ 3-fp (Fig. 3C) demonstrates that a nephritogenic epitope of HN is located within the N-terminal 86 amino acids of the 44-kDa subunit of HNAC (Fig. 3C).

Antibody to the C14/ Δ 3-fp Induces IDs. When anti-gp330 IgG was injected into normal rats, numerous relatively large granular IDs typical of HN were detected in the glomeruli after 3 days (Fig. 4A). When the same amount of anti-C14-fp IgG was injected, similar IDs were observed except, as reported (12), they were smaller and less numerous than those induced by anti-gp330 IgG (Fig. 4B). In addition, some mesangial binding of the anti-C14-fp antibody was regularly observed. Injection of IgG specific for each of the deletion clones resulted in a pattern of IDs indistinguishable from that found with the entire anti-C14-fp IgG (Fig. 5A). In contrast, when anti-C14-fp IgG was immunodepleted of its reactivity toward the C14/ Δ 3-fp and injected, virtually no glomerular IDs were observed (Fig. 5B), despite the fact that an $\approx$ 100-fold excess of IgG was administered. The fact that the ID-forming potency was retained by the fp of the smallest deletion clone (C14/ Δ 3) indicates that at least one epitope is contained within the 86 amino acids of the C14/ Δ 3-fp. We conclude that (i) The C14/ Δ 3-fp domain contains a major pathogenic epitope of HN, and (ii) no other additional pathogenic epitopes are present on the C14-fp. Injection of IgG specific for *E. coli* β -galactosidase resulted in a faint mesangial pattern typical of nonspecific IgG injection (data not shown).

DISCUSSION

In this report, we have identified an 86-amino acid polypeptide domain contained within the 44-kDa subunit of HNAC

that constitutes a pathogenic epitope responsible for the initiation of HN. Characterization of this epitope involved stepwise truncation of the 3' end of the C14 cDNA sequence encoding the 44-kDa polypeptide and subsequent expression of the deletion clones as fusion proteins. The shortest fusion protein, C14/ Δ 3-fp, representing the N-terminal 86 amino acids of the 44-kDa subunit, specifically bound a subfraction of the anti-C14-fp IgG. When injected into rats, the IgG that bound to the C14/ Δ 3-fp resulted in induction of glomerular IDs, whereas the C14/ Δ 3-fp-depleted IgG was negative. Thus, our results demonstrate that a major nephritogenic or pathogenic epitope of HNAC is encoded by the N-terminal 86 amino acids of the C14 peptide. The term pathogenic epitope operationally defines an amino acid sequence presented in the native antigen that is accessible and is capable of binding circulating antibodies *in vivo*, leading to the formation of glomerular IDs (3, 4, 10, 12).

A limitation of our approach is that antibodies were generated against SDS-denatured, electroeluted fp and injected into rats, where antigenic sites are presented in a native state. Antigenic epitopes are often formed by structural combinations consisting of α -helices, β -sheets, and β -turns of the native molecule arranged in a discontinuous fashion (13–16). The stability of these conformations is therefore expected to be perturbed by denaturing conditions—for example, by SDS. In the epitope analysis of the HN antigen, identification of the pathogenic epitope is based on the unique ability of the epitope-specific antibody to induce glomerular IDs when injected *i.v.*, which constitutes a highly sensitive and functionally relevant *in vivo* assay. Based on the findings reported here it can be concluded that the 86 amino acids of the recombinant C14/ Δ 3-fp resemble the antigenic conformation of the native epitope in the C14-fp, because specific antibodies are able to recognize the antigen and bind *in vivo*. It will be of interest to find out whether smaller fragments of the C14/ Δ 3 peptide will be effective in initiating ID formation.

Our earlier studies suggest that antibody binding to coated pits at the base of the glomerular epithelium induces confor-

FIG. 5. Detection by direct immunofluorescence of rabbit IgG injected 3 days previously into rats. (A) Glomerulus of a rat that received $\approx$ 50 μ g of IgG specific for the C14/ Δ 3-fp that had been affinity purified from 4 ml of anti-C14-fp antiserum. There are small IDs in the peripheral capillary loops (arrowheads), and some IgG is also found in the mesangium (M). (B) Glomerulus of a rat injected with $\approx$ 4 mg of affinity-purified anti-C14-fp IgG that had been immunodepleted of its reactivity with C14/ Δ 3-fp. Despite the huge excess of IgG compared to that used in A, few granular deposits are found in the GBMs, and there are no IgG deposits in the mesangium. L, capillary lumen; US, urinary space. ($\times$ 900.)

mational changes in the antigen, resulting in shedding of the immune complexes and their attachment via the antigen to the glomerular basement membrane (GBM) (17). In this context, it is of interest that the 44-kDa polypeptide encoded by the C14 cDNA, now known to form a heterodimer with gp330 (3), shares >90% sequence identity with HBP-44, a heparin binding protein isolated from a mouse teratocarcinoma cell line (18). Thus, binding of the 44-kDa subunit to heparan sulfate moieties of the heparan sulfate proteoglycans present in the lamina rara externa of the GBM (19) may play an important role in immobilization of immune complexes to the GBM and subsequent disruption of its integrity. Additional epitopes or cryptotopes exposed during immune complex formation may play a role in the growth of IDs as other antigenic determinants become available for antibody binding. It might be anticipated that an antiserum that contains IgGs specific for a single pathogenic epitope might induce nascent IDs of small size, because there should be no binding of additional IgGs with reactivity to additional epitopes or cryptotopes. The fact that the IDs that result from the injection of anti-C14/ Δ 3-fp IgG are smaller than those induced by anti-C14-fp IgG is compatible with this view.

The ultimate goal for the study of models of autoimmune diseases is to use the information obtained to attempt to develop therapeutic strategies. A prerequisite for the design of specific therapies is detailed knowledge of the structure of the antigenic epitopes involved. A number of these structures have been identified—for example, in the myelin basic protein in allergic encephalitis (20), the retinal S protein in uveitis (2), and the acetylcholine receptor complex in myasthenia gravis (21). The number of pathogenetically relevant epitopes in a given antigen to which circulating antibodies can bind appears to be rather limited, even on large proteins, as exemplified by the fact that there is but a single main immunogenic region (residues 67–76) of the α subunit of the large acetylcholine receptor complex (21) responsible for the pathogenesis of myasthenia gravis. Attempts to interfere with autoimmune diseases have been based on strategies that involve specific modification of immune responses at several levels. One such strategy is the use of synthetic peptides that mimic the pathogenic epitopes (22–26). Similar strategies to interfere with glomerular autoimmune diseases are not yet available because their molecular pathogenesis has not been sufficiently characterized.

In the future it will be of interest to study the structure of the pathogenic epitope(s) on the C14/ Δ 3-fp in even greater detail, because this simple system may represent a model for the general principles that govern the formation of IDs in passive HN and presumably also in human membranous nephropathy. It will also be of interest to identify and map additional epitopes—other than that found on the C14/ Δ 3 polypeptide—on the gp330–44-kDa HNAC complex. It has been suggested that gp330 may belong to the LDL receptor family (8) because it shares regions of homology with the LDL and LRP receptors (27). It also shares with the LRP receptor the ability to bind the 44-kDa receptor-associated protein (3, 7). At present, sequence information on gp330 is fragmentary, and therefore complete mapping of pathogenic epitopes on the gp330–44-kDa complex will be possible only when the complete amino acid sequence of gp330 is available.

This work was supported by National Institutes of Health Grant DK17724 to M.G.F. and Grant P7742Med from the Fonds zur Förderung der Wissenschaftlichen Forschung to D.K. R.A.O. was supported by Public Health Service Award NS07078-13.

1. Couser, W. G. (1986) in *Textbook of Medicine. A Systematic Approach*, ed. Stein, J. (Brown-Little, Boston), pp. 834–861.
2. Thureau, S. R., Chan, C. C., Suh, E. & Nussenblatt, R. B. (1991) *Autoimmunity* 4, 507–516.
3. Orlando, R. A., Kerjaschki, D., Kurihara, H., Biemesderfer, D. & Farquhar, M. G. (1992) *Proc. Natl. Acad. Sci. USA* 89, 6698–6702.
4. Pietromonaco, S. D., Kerjaschki, S., Binder, R., Ullrich, R. & Farquhar, M. G. (1990) *Proc. Natl. Acad. Sci. USA* 87, 1811–1815.
5. Kounnas, M. Z., Morris, R. E., Thompson, M. R., FitzGerald, D. J., Strickland, D. K. & Saelinger, C. B. (1992) *J. Biol. Chem.* 267, 12420–12423.
6. Kristensen, T., Moestrup, S. K., Glieman, J., Bendtsen, L., Sand, O. & Sottrup-Jensen (1990) *FEBS Lett.* 276, 151–155.
7. Strickland, D., Ashcom, J. D., Williams, S., Burgess, W. H., Migliorini, M. & Argraves, S. M. (1990) *J. Biol. Chem.* 265, 17401–17404.
8. Brown, M. S., Herz, J., Kowal, R. C. & Goldstein, J. L. (1991) *Curr. Opin. Lipidol.* 2, 65–72.
9. Henikoff, S. (1987) *Methods Enzymol.* 155, 156–165.
10. Kerjaschki, D. & Farquhar, M. G. (1982) *Proc. Natl. Acad. Sci. USA* 79, 5557–5561.
11. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) in *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
12. Kerjaschki, D. & Farquhar, M. G. (1983) *J. Exp. Med.* 157, 667–686.
13. Amit, A. G., Mariuzza, R. A., Phillips, S. E. V. & Poljak, R. J. (1986) *Science* 233, 747–753.
14. Sheriff, S., Silverton, E. W., Padlan, E. A., Cohen, G. H., Smith-Gill, S. J., Finzel, B. C. & Davies, D. R. (1987) *Proc. Natl. Acad. Sci. USA* 84, 8075–8079.
15. Padlan, E. A., Silverton, E. W., Sheriff, S., Cohen, G. H., Smith-Gill, S. J. & Davies, D. R. (1989) *Proc. Natl. Acad. Sci. USA* 86, 5938–5942.
16. Tulip, W. R., Varghese, J. N., Webster, R. G., Air, G. M., Laver, W. G. & Colman, P. M. (1990) *Cold Spring Harbor Symp. Quant. Biol.* 54, 257–263.
17. Kerjaschki, D., Miettinen, A. & Farquhar, M. G. (1987) *J. Exp. Med.* 166, 109–128.
18. Furukawa, T., Ozawa, M., Huang, R.-P. & Muramatsu, T. (1990) *J. Biochem.* 108, 297–302.
19. Farquhar, M. G. (1991) in *Cell Biology of Extracellular Matrix, Second Edition*, ed. Hay, E. D. (Plenum, New York), pp. 365–418.
20. Hashim, G. A. & Day, E. D. (1988) *J. Neurosci. Res.* 21, 1–5.
21. Tzartos, S. J., Kolka, A., Walgrave, S. L. & Conti-Tronconi, B. M. (1988) *Proc. Natl. Acad. Sci. USA* 85, 2899–2903.
22. Roberge, F. G., Lorberboum-Galski, H., Le Hoang, P., de Smet, M., Chan, C., Fitzgerald, D. & Pastan, I. (1989) *J. Immunol.* 143, 3498–3502.
23. Kumar, V., Urban, J. L., Horvath, S. J. & Hood, L. (1990) *Proc. Natl. Acad. Sci. USA* 87, 1337–1341.
24. Steinman, L. (1991) *Adv. Immunol.* 49, 357–379.
25. Smilek, D. E., Lock, C. B. & McDevitt, H. O. (1990) *Immunol. Rev.* 118, 37–71.
26. Talal, N. (1989) *J. Autoimmun.* 2, 257–264.
27. Raychowdhury, R., Niles, J. L., McCluskey, R. T. & Smith, J. A. (1989) *Science* 244, 1163–1165.