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Distinct Profiles of Immunoglobulin G-Binding-Protein Expression by Invasive Serotype M1 Isolates of *Streptococcus pyogenes*

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Analysis of immunoglobulin G (IgG)-binding-protein expression by invasive group A streptococcal isolates of the M1 serotype collected as part of a Centers for Disease Control and Prevention surveillance study revealed two distinct phenotypes. One group of type M1 isolates expressed a surface protein reactive with all four human IgG subclasses (type IIo), while a second group expressed a surface protein demonstrating significant reactivity only with human IgG3 (type IIb). The functional forms of IgG-binding protein were antigenically related, and both were recognized by a rabbit polyclonal antiserum to serotype M1 but not by normal rabbit serum. While the quantities of antigenic M1 protein present in the extracts of representative isolates displaying each phenotype differed, the functional differences were found to be qualitative and not solely quantitative. The IgG-binding properties of these antigenically related M1 proteins could be readily distinguished from those of another IgG-binding protein, protein H. Type M1 isolates of the IIb phenotype differed from those of the IIo phenotype by secreting larger amounts of a casein-hydrolyzing protease into culture supernatants.

In the mid-1980s a resurgence of severe group A streptococcal disease, including a previously unknown toxic-shock-like syndrome, occurred worldwide in immunocompetent patients with no known predisposing factors (2, 3, 5, 6, 12, 13, 15, 18, 19, 27–29). The predominant serotype associated with the recent outbreak of severe invasive disease was type M1. Analysis of immunoglobulin G (IgG)-binding proteins expressed by invasive group A streptococcal isolates, collected as part of a Centers for Disease Control and Prevention (CDC) (Atlanta, Ga.) surveillance study (30), revealed an unexpected difference among isolates of the M1 serotype (24).

Two distinct phenotypes of M1 isolates could be identified on the basis of the profile of IgG-binding proteins present in CNBr extracts. Extracts from one group of isolates contained IgG-binding proteins reactive with all four human IgG subclasses, while extracts of the second group demonstrated IgG-binding proteins reactive preferentially with human IgG3. This finding was of particular interest because of recent reports that the M1 gene product itself was an IgG-binding protein (4, 24, 26) and because of other studies with a mouse model of skin infection that suggested an association between IgG-binding-protein expression and invasive potential (22, 23). Certain M1 isolates have been found to express another IgG-binding protein, protein H (1, 10), in addition to the M1 gene product.

Protein H binds all four human IgG subclasses in a nonimmune fashion and has been purified from M1 isolate AP1, cloned, and sequenced (1, 10). The gene encoding protein H is found exclusively in M1 isolates and is located on the chromosome adjacent to the *emmL1* gene (1). There is evidence to suggest that these two genes are related and most probably arose through a gene duplication event. The studies described in this paper were designed to analyze IgG-binding proteins expressed by isolates with distinct IgG-binding phenotypes associated with serotype M1 to determine if these phenotypic differences could be explained by selective expression of the protein H gene in addition to the M1 IgG-binding gene product.

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MATERIALS AND METHODS

Bacteria. Group A streptococcal isolates were obtained from the CDC. These isolates have been extensively characterized at the CDC for a variety of phenotypic and genotypic characteristics (30). The immunological status of the patients, disease course, and outcome of the infection have all been well characterized as part of this surveillance study. All isolates were stored as glycerol stocks at −70°C, and bacteria utilized in these studies were grown from the original stocks.

Solubilization of IgG-binding proteins from group A streptococcal isolates. All isolates were grown overnight at 37°C as stationary-phase cultures in Todd-Hewitt broth. Approximately 2 g (wet weight) of bacteria per liter was recovered. IgG-binding proteins were solubilized by CNBr treatment as detailed previously (25). Briefly, bacteria from an overnight culture in Todd-Hewitt broth were sedimented by centrifugation at 4,000 × *g*, washed in phosphate-buffered saline (PBS; 0.01 M, pH 7.4), and resuspended to 25% (wt/vol) in PBS. Equal volumes of CNBr (30 mg/ml in 0.2 N HCl, pH 2.0) were added to each suspension. Following a 16-h rotation at ambient temperature, bacteria were sedimented by centrifugation and the bacterium-free supernatant was dialyzed extensively against several changes of 0.1 N HCl. Extracts were neutralized by the addition of 1.5 M Tris HCl, pH 8.8, and clarified by passage through a 0.2-μm-pore-size filter.

Plasma proteins. Human IgG myeloma proteins of each subclass were obtained from the immunoglobulin subcommittee of the World Health Organization/International Union of Immunologic Societies. Human serum albumin, fibrinogen, and serum IgA were obtained from Organon Teknika Corporation, Durham, N.C.

Labeling of proteins. Proteins were radiolabeled with ^{125}I (Amersham Corp., Chicago, Ill.) by a lactoperoxidase method (31). A PD-10 desalting column (Pharmacia Fine Chemicals, Piscataway, N.J.) was used to separate labeled proteins from free iodine. The specific activity of all radiolabeled proteins was approximately 0.3 mCi/mg.

Polyacrylamide gel electrophoresis (PAGE) and Western blotting (immunoblotting) techniques. Protein samples were denatured by boiling for 5 min in 0.5 M Tris-HCl (pH 6.8) containing 2% (wt/vol) sodium dodecyl sulfate (SDS), 5% (vol/vol) β-mercaptoethanol, 10% (vol/vol) glycerol, and 0.01% (wt/vol) bromphenol blue. Denatured proteins were electrophoresed in 10% polyacrylamide slab gels at 50 V for 16 h according to the method of Laemmli (16). Prestained molecular weight standards (Bio-Rad, Richmond, Calif.) containing phosphorylase *b* (M_r, ~106,000), bovine serum albumin (M_r, ~80,000), ovalbumin (M_r, ~49,500), carbonic anhydrase (M_r, ~33,500), soybean trypsin inhibitor (M_r, ~27,500), and lysozyme (M_r, ~18,500) were included in each SDS-polyacrylamide gel assay.

The proteins were electrophoretically transferred to nitrocellulose (Bio-Rad) by a modification of the method described by Towbin et al. (32). Briefly, SDS-polyacrylamide slab gels were presoaked for 30 min in 25 mM Tris–192 mM glycine–20% methanol (pH 8.3), assembled into a high-field-intensity transblot system (Bio-Rad), and electrophoresed in the same buffer at 70 V for 3 h. The nitrocellulose blots were washed four times with 250 ml of 50 mM Veronal buffer, pH 7.4 (VBS), containing 0.15 M NaCl, 0.25% gelatin, and 0.25% Tween 20 (VBS-gelatin-Tween), to saturate the remaining protein-binding sites on the nitrocellulose. Each wash was carried out for 15 min at ambient temperature with agitation. The nitrocellulose membranes were incubated for 3 h at ambient temperature in 25 ml of VBS-gelatin-Tween containing 3×10^5 cpm of the appropriate ^{125}I-labeled probe per ml. The nitrocellulose membranes were then washed four times (for 15 min each time) with 250 ml of VBS containing 0.01 M ethylenediaminetetraacetate (pH 7.2), 1 M NaCl, 0.25% gelatin, and 0.25% Tween 20 on a rocking platform at ambient temperature. The membranes were dried and autoradiographed with Kodak XAR-5 film and Kodak X-Omat intensifying screens at −70°C for 1 to 3 days.

Competitive ELISA to determine functional IgG3-binding activities. Competitive enzyme-linked immunosorbent assay (ELISA) was carried out as previously described by Pack et al. (21). Form 1 and form 2 IgG3-binding proteins from representative group A strains A992 (M18) and 529 (M12), respectively, were biotinylated and used as tracers in the assay. Preliminary checkerboard studies were carried out to establish the following: (i) the appropriate level of antigen for coating the plate (100 ng of IgG3 per well), (ii) the optimal dilution of the biotinylated tracer (1:1,800 for the 529 tracer and 1:7,500 for the A992 tracer), and (iii) the optimal dilution of streptavidin-alkaline phosphatase (1:3,000) for enabling IgG3-binding-protein tracer activity to be quantified.

Once these conditions were established, the ELISA was conducted by combining 100 μl of a sample containing a CNBr extract of a selected group A streptococcus with 50 μl of an appropriate dilution of biotinylated tracer in a microtiter well coated with IgG3 (cryo)myeloma protein (21). Following an incubation period of 1 h at ambient temperature, the microtiter plates were washed six times with 10 mM Tris-buffered saline, pH 8.0, containing 0.01% Tween 20. The quantity of bound tracer was determined by incubation with 100 μl of a 1:3,000 dilution of streptavidin-alkaline phosphatase (Bio-Rad) for 1 h at ambient temperature, followed by washing and subsequent incubation with an appropriate substrate (1 mg of *p*-nitrophenyl phosphate [Sigma Diagnostics, St. Louis, Mo.] per ml in 1 M diethanolamine-HCl [pH 9.8] containing 0.5 mM $MgCl_2$). The A_{405} was measured over time with a Biokinetics reader (BioTek Instruments, Winooski, Vt.).

In this assay, form 1 IgG3-binding proteins inhibit the binding of the A992-biotinylated tracer but not of the 529-biotinylated tracer, while form 2 IgG3-binding protein displayed the opposite inhibitory pattern (21).

Assays for protease production. Protease production in culture supernatants of type M1 isolates was determined by a modification of the method described by Elliott (7). Type M1 isolates were grown for 24 h at 37°C in the chemically defined medium for streptococci described by van de Rijn and Kessler (33) containing a 10% filtrate of Todd-Hewitt broth which was passed through a YM-10 filter with an M_r cutoff of ~10,000 (Amicon, Beverly, Mass.). Bacteria were removed by centrifugation, and the culture supernatant was brought to 70% saturation with ammonium sulfate. Precipitated material was recovered by centrifugation at 4,000 × *g* for 20 min, dissolved in a volume of distilled water equivalent to a 50% (wt/vol) suspension of bacteria recovered from the original culture, and dialyzed extensively against distilled water. Protease activity was determined by adding 250 μl of this material to 250 μl of 0.1 M sodium phosphate buffer, pH 7.5, in the presence or absence of 200 mM 2-mercaptoethanol. Following a 1-h incubation at 37°C, 500 μl of 1% casein solution in PBS was added to each tube. Hydrolysis of casein was determined by the addition of 100 μl of the reaction mixture to 900 μl of ice-cold 5% trichloroacetic acid at time zero and at hourly intervals thereafter. Casein hydrolysis was shown to be proportional to the time of incubation over the 5-h assay period. The undigested trichloroacetic acid-precipitable protein was pelleted by centrifugation, and the A_{280}s of the supernatants were determined.

RESULTS

Evidence for heterogeneity in IgG-binding-protein patterns of extracted proteins from group A isolates of the M1 serotype. Ten group A M1 isolates from the CDC surveillance studies (30) were selected for analysis. The characteristics of these isolates are presented in Table 1. Five of these isolates expressed IgG-binding proteins of the type IIo form (reactive with all four human IgG subclasses), and five isolates expressed immunoglobulins of the type IIb form (reactive preferentially with human IgG3). In the initial experiments, selected isolates were grown overnight as stationary-phase cultures in Todd-Hewitt broth, and CNBr extracts were prepared as described in Materials and Methods. Preliminary experiments were carried out to determine the level of IgG-binding-protein activity in the CNBr extracts of representative isolates.

TABLE 1. Characteristics of selected serotype M1 group A streptococcal isolates

Isolate designation	IgG-binding-protein type[a]	Production of toxin[b]:		Positive for STSS[c]
		A	B	
823	IIo	+	+	No
906	IIo	+	−	Yes
1148	IIo	+	−	No
1154	IIo	+	+	Yes
2110	IIo	+	−	Yes
389	IIb	−	−	Yes
1054	IIb	+	+	Yes
1143	IIb	−	+	Yes
1269	IIb	−	+	Yes
1881	IIb	−	−	No

[a] Type IIo molecules bind all four human IgG subclasses. Type IIb molecules preferentially bind human IgG3 (23).
[b] Determined by the CDC by immunoblotting (28).
[c] Patients with streptococcal toxic-shock-like syndrome (STSS) met the criteria for this condition as defined by the Working Group on Severe Streptococcal Infections (34).

Extracts containing approximately equivalent levels of IgG-binding protein were separated on parallel SDS-polyacrylamide gels, electroblotted to nitrocellulose, and probed with ^{125}I-labeled human IgG1 (Fig. 1a), ^{125}I-labeled human IgG3 (Fig. 1b), or a rabbit anti-serotype M1 antiserum followed, after a washing step, by ^{125}I-labeled protein G (Fig. 1c). The results of the studies presented in Fig. 1 demonstrate that there was an apparent difference in the functional binding profiles of the extracts (Fig. 1a and b). Five of the extracts contained an IgG-binding protein reactive with IgG1 and IgG3, while five of the extracts contained proteins reactive with human IgG3 but not IgG1 under identical experimental probing conditions. The proteins binding IgG1 were also found to bind IgG2 and IgG4 (type IIo reactivity), while the proteins binding IgG3 that failed to react with IgG1 also failed to react with either IgG2 or IgG4 (data not shown). None of the extracts demonstrated significant reactivity with normal rabbit serum under the conditions tested (data not shown).

The extracts containing the greatest quantity of antigenic M1 protein bound all four human IgG subclasses, while those containing lower concentrations of antigenic protein preferentially bound IgG3 (Fig. 1b). In all cases, the immunoglobulin-binding proteins were recognized by an anti-serotype M1 antiserum (Fig. 1c). In general, extracts of the type IIo-expressing isolates contained two major forms of reactive molecules (M_rs, ~47,000 and ~49,000), while extracts from isolates that expressed type IIb activity contained a single predominant form (M_r, ~47,000) of the IgG-binding molecule.

Analysis of representative antigenic M1 proteins for IgG1-binding relative to IgG3-binding activity. The differences in the immunoglobulin-binding profiles between the group A isolates could be quantitative rather than qualitative, i.e., the detection of IgG3 binding might be more sensitive and thus IgG3 binding in the absence of IgG1 binding might occur at low concentrations of the antigenic M1 protein. Consequently, a series of experiments were performed with a representative isolate that bound all four subclasses (isolate 906) and a putative IgG3 preferential binder (isolate 1881). Dilutions of cyanogen bromide extracts of each organism were separated by SDS-PAGE, electroblotted to nitrocellulose, and probed with

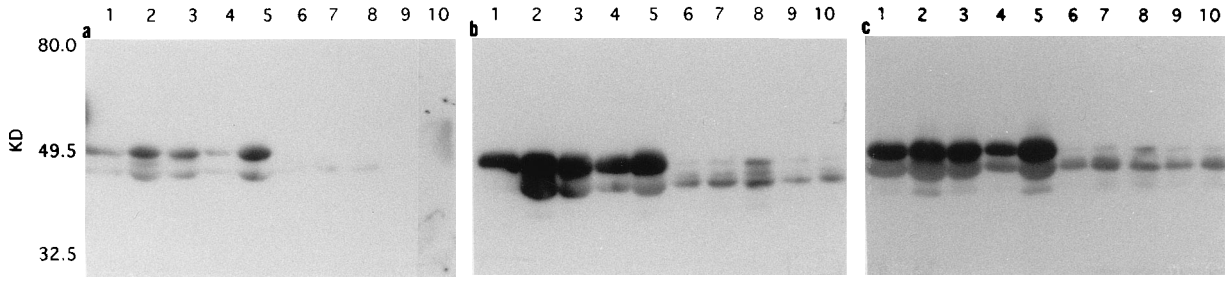

FIG. 1. Antigenic analysis of IgG-binding proteins in CNBr extracts of selected serotype M1 group A streptococcal isolates. CNBr extracts of selected type M1 group A isolates were grouped according to the pattern of IgG-binding reactivity observed in preliminary experiments. The first five lanes contained extracts that reacted with all four human IgG subclasses, and the second five lanes contained extracts reactive predominantly with IgG3. Each sample was separated in an SDS–10% polyacrylamide gel under reducing conditions and electroblotted to nitrocellulose. Unreactive sites were blocked, and the membranes were probed with either radioiodinated human IgG1 (a) or radioiodinated human IgG3 (b), or the membranes were probed in a sandwich-type assay involving a rabbit anti-serotype M1 antiserum followed, after a washing step, by radioiodinated protein G (c). The probed blots, after being washed, were exposed to X-ray film with an intensifying screen for 24 to 48 h at −70°C. A parallel blot probed with normal rabbit serum followed by radioiodinated protein G demonstrated no reactive bands (data not shown).

either radiolabeled human IgG1 or IgG3 or probed antigenically in a sandwich assay involving rabbit anti-serotype M1 antiserum followed by ^{125}I-labeled protein G. Autoradiographs were developed, and the activities of reactive bands were determined by densitometry.

Correlation curves for antigenically reactive proteins present in an extract and binding of either IgG1 or IgG3 were developed (Fig. 2). These results demonstrate that extracts of both isolate 906 and isolate 1881 bound IgG3 in a manner related to the concentration of epitopes recognized by the serotype M1 antiserum (Fig. 2a); however, IgG1 binding was found only for antigenic proteins from extracts of isolate 906 (Fig. 2b). The concentration of IgG1 bound by extracts of strain 906 was directly related to the concentration of IgG3 bound, while there was no relationship between these functional properties in extracts of strain 1881 (Fig. 2c). Consequently, the difference in binding to antigenically related M1 proteins in the extracts was qualitative.

Analysis of representative group A isolates for expression of protein H. Recent studies have shown that certain M1 isolates express an IgG-binding protein, protein H, reactive with all four human IgG subclasses (10). The protein H gene shows extensive sequence similarity to the *emm*(-like)*1* gene and most probably arose through a gene duplication event (1, 10). The possibility exists that the observed differences between type IIo- and type IIb-expressing M1 isolates might be explained by differential expression of two independent genes, i.e., an *emm*(-like)*1* gene coding for a type IIb protein and a protein H gene coding for a type IIo binding protein.

PCR analysis of the *vir* regulon of representative type M1 isolates 1881 and 906 demonstrated the presence of a protein H gene in the chromosomal DNA of both isolates (data not shown). This finding raised the possibility that under certain conditions, bacteria could express either protein H alone, M1 alone, or both gene products. Since protein H is known to be a type IIo binding protein (10), this might account for the activity observed in extracts of isolate 906. This possibility was tested directly in the next series of studies.

The IgG-binding properties of extracts of the prototype M1 isolate from which protein H was first isolated, AP1, were compared with those of CNBr extracts of a representative type IIo-expressing M1 isolate (906). Recombinant protein H (a kind gift from Lars Björck) was also included as a reference positive control. The results presented in Fig. 3 demonstrate a distinct pattern of IgG-binding bands associated with CNBr extracts of strain AP1 compared with the pattern extracts of the type IIo binding protein, M1 isolate 906. Three IgG-binding protein bands were detected with extracts of AP1. Two of

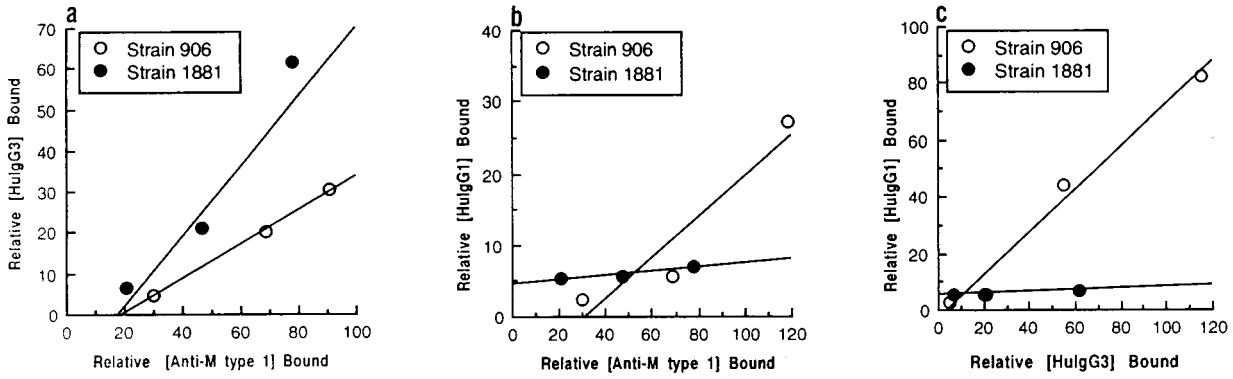

FIG. 2. Relationship between binding of IgG1 or IgG3 and the quantity of antigenic M1 protein in CNBr extracts of two representative M1 isolates. Twofold dilutions of CNBr extracts of a representative type IIo-expressing isolate (strain 906) and a representative type IIb-expressing isolate (strain 1881) were separated in SDS–10% polyacrylamide gels under reducing conditions and electroblotted to nitrocellulose. Unreactive sites were blocked, and the membranes were probed with either radioiodinated human IgG1 (HuIgG1) (b and c) or radioiodinated human IgG3 (HuIgG3) (a and c), or they were probed in a sandwich assay with rabbit anti-serotype M1 antiserum (anti-M type 1) (a and b) followed, after a washing step, by radiolabeled protein G. The probed blots were washed and exposed to X-ray film with an intensifying screen for 16 to 48 h at −70°C. The area of reactive bands on each autoradiograph was determined by densitometry. The correlation curves were generated for each probe to relate the relative intensities of the bands on the autoradiograph to the concentrations of samples applied to the gel.

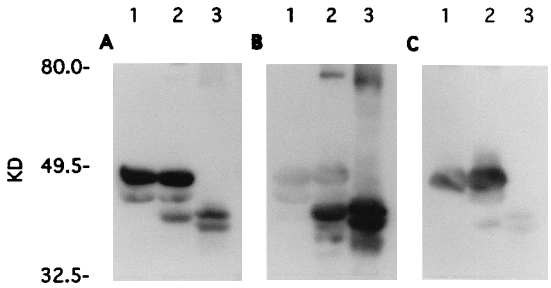

FIG. 3. Comparison of IgG-binding proteins in CNBr extracts of representative type IIo IgG-binding group A streptococcal isolates with those of the protein H-expressing prototype M1 isolate, AP1. Lane 1 contains the CNBr extract of a representative type IIo-expressing M1 isolate, 906. Lane 2 contains the CNBr extract of the prototype protein H-expressing serotype M1 group A isolate, AP1, and lane 3 contains recombinant protein H. Samples were separated in 10% polyacrylamide gels under reducing conditions and electroblotted to nitrocellulose. Unreactive sites were blocked, and the membranes were probed with either radioiodinated human IgG1 (A) or radioiodinated human IgG3 (B), or the membranes were probed in a sandwich assay with rabbit anti-serotype M1 serum followed, after a washing step, by radioiodinated protein G (C). The probed blots were washed and exposed to X-ray film with an intensifying screen for 16 to 48 h at −70°C.

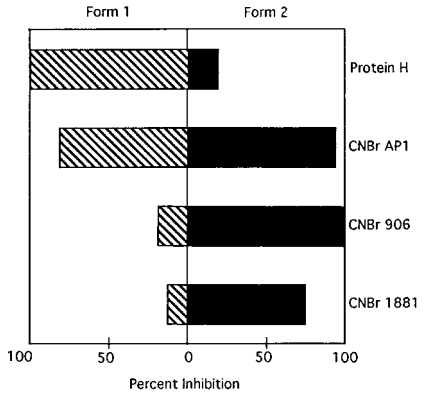

FIG. 4. Analysis of the functional IgG3-binding activity in extracts of representative serotype M1 group A isolates. CNBr extracts of a representative type IIo M1 isolate (906), a representative type IIb isolate (1881), and the prototype protein H-expressing M1 isolate (AP1) were used to inhibit the binding of form 1 or form 2 biotinylated probes to human IgG3 by the competitive ELISA described in Materials and Methods. Recombinant protein H was included as a reference control.

these protein bands were detected antigenically with an anti-serotype M1 antiserum followed by ^{125}I-labeled protein G and migrated to a molecular weight equivalent to those present in the extract of strain 906. The unique lower-molecular-weight IgG-binding protein present only in the AP1 extract migrated to a molecular weight equivalent to that of recombinant protein H and, like protein H, reacted with human IgG1 and IgG3 (Fig. 3A and B), as well as with human IgG2 and IgG4 (data not shown). Recombinant protein H demonstrated weak reactivity with the anti-serotype M1 antiserum relative to IgG-binding activity under identical probing conditions (Fig. 3C). This most probably reflects the presence of a shared epitope, which might be expected since the protein H gene most probably arose from the M1 gene by a duplication event (1).

Recent studies from our laboratory have demonstrated that group A streptococci can express two distinct forms of functional IgG3-binding protein (20, 21). One form binds to all IgG3 myelomas tested and targets a site on IgG3 similar to that targeted by streptococcal protein G (form 1). The second functional IgG3-binding form reacts with a limited number of IgG3 myelomas and binds to a site on the IgG3 molecule distinct from that recognized by protein G (form 2). By comparing the inhibitory potentials of extracts of group A streptococci in a competitive ELISA, these two functional reactivities can be distinguished readily (20, 21). We have carried out these studies with extracts from the protein H-expressing isolate (AP1), the type IIo-expressing M1 strain (906), and the type IIb-expressing M1 strain (1881) and with recombinant protein H.

Extracts of M1 isolates 906 and 1881 demonstrated the presence of a form 2 IgG3-binding protein (Fig. 4). By contrast, recombinant protein H failed to display any form 2 IgG3-binding activity but demonstrated potent form 1 IgG3-binding activity. Extracts of group A isolate AP1 were found to be capable of mediating inhibition in both IgG3 functional assays, indicating the presence of both a form 1 and a form 2 IgG3-binding protein activity.

Taken together, these studies indicate that protein H is a form 1 IgG3-binding protein which reacts with all four human IgG subclasses. By contrast, the two functional forms of IgG-binding proteins recognized by the anti-serotype M1 antiserum both expressed form 2 IgG3-binding activity. Consequently, the properties of the M1(-like) IgG-binding proteins could be readily distinguished from those of protein H and would not account for the differences in the IgG-binding profiles of the two functional variants of M1 isolates observed in this study.

Comparison of levels of protease production by M1 isolates expressing distinct immunoglobulin-binding proteins. In the prior characterization of the M1 isolates used in this study, the CDC reported an association between production of a case-in-hydrolyzing protease and isolates causing soft tissue necrosis (30). In the next series of experiments, the protease production by serotype M1 isolates representing the different IgG-binding phenotypes was tested, as described in Materials and Methods, to see if these two properties were associated. Enzymatic activity was monitored over time. The results for the 5-h time point are presented in Fig. 5 and demonstrate that those isolates expressing the type IIb form IgG-binding protein hydrolyzed a greater quantity of casein than those isolates expressing the type IIo phenotype.

DISCUSSION

Analysis of IgG-binding-protein expression by M1 isolates collected as part of a CDC surveillance study of the recent

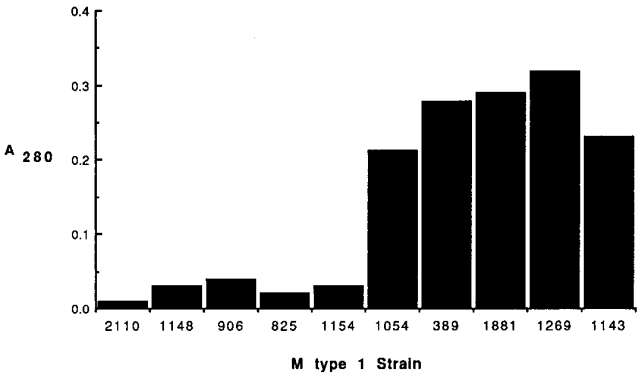

FIG. 5. Analysis of protease production by selected serotype M1 (M type 1) group A isolates. Culture fluids from representative isolates were tested, following reduction, for the ability to hydrolyze casein. Enzymatic activity was determined by measuring the generation of trichloroacetic acid-soluble peptides from casein as described in Materials and Methods.

resurgence of severe group A streptococcal infections and a toxic-shock-like syndrome identified two profiles of IgG-binding-protein expression associated with M1 isolates (24). One group of type M1 isolates expressed a surface protein reactive with all four human IgG subclasses (type IIo), while a second group expressed a surface protein demonstrating significant reactivity only with human IgG3 (type IIb). Evidence for at least two IgG-binding proteins in group A isolates of the M1 serotype at both the gene and the protein level has previously been reported (1, 8, 10). Schmidt and Wadström (26) and our laboratory (4, 24) have provided evidence that the M1 protein itself can bind immunoglobulin in a nonimmune manner. In addition, Björck and colleagues identified a unique type IIo IgG-binding protein, protein H, expressed on type M1 group A streptococcal isolate AP1 (1, 8, 10).

The gene encoding protein H is found in the *vir* regulons of the majority of M1 isolates that have been studied, and these genes are located next to each other on the chromosome (1). Since the protein H gene is closely related to the M1 gene and most probably arose by a gene duplication event, the contribution of protein H to the immunoglobulin-binding phenotype was assessed. The present study was designed to characterize the IgG-binding-protein expression of representative M1 isolates expressing distinct profiles of IgG-binding activity to determine if these differences were related to changes in the properties of a single protein or were attributable to distinct gene products.

Representative M1 isolates were extracted by treatment with CNBr, and the solubilized proteins were compared in a series of functional and antigenic assays. Extracts of M1 isolates that bound all four human IgG subclasses contained a greater quantity of antigenic M1 protein, and this activity was associated with two major proteins with M_rs of ~47,000 and ~49,000. The second profile of extracted proteins, which reacted preferentially with human IgG3, contained less M1 antigenic protein and demonstrated only the lower-molecular-weight form (M_r, ~47,000) of the protein. Comparison of functional IgG-subclass-binding activity for equivalent M1-antigenic levels of extracted protein showed that the difference in IgG-binding activity was qualitative and not solely quantitative (Fig. 2).

The observed differences in the molecular sizes of reactive bands raised the possibility that the expression of more than one gene product, in particular, the protein H gene product, might contribute to the IgG-binding-protein profiles. Analysis of recombinant protein H demonstrated that this protein was functionally and antigenically distinct from the IgG-binding proteins associated with extracts of either phenotypic family of M1 isolates. In particular, the IgG3-binding-protein profiles of protein H and the M1 extracts were distinct. Protein H was shown to bind IgG3 in a manner analogous to that of protein G (form 1), while the M1 extracts failed to display form 2 IgG3-binding activity, i.e., non-protein G-inhibitable binding activity. It was of interest that the prototype M1 isolate, AP1, from which protein H was first isolated and cloned contained both form 1 (protein H) and form 2 (M1-like protein) IgG3-binding proteins (Fig. 4). None of the clinical isolates studied contained detectable protein H-like functional activity in CNBr extracts. Taken together, all of these studies confirm the existence of two IgG-binding forms of M1-like proteins.

Examination of laboratory and clinical data provided by the CDC for the M1 strains analyzed in this study showed some differences in other virulence factors (Table 1). All M1 isolates expressing type IIb binding proteins (IgG3 only) produced significantly higher levels of protease than the isolates expressing IgG-binding proteins reactive with all four IgG subclasses. The protease production was not restricted to isolates shown to produce streptococcal pyogenic exotoxin B, a known cysteine protease, and may, therefore, represent the activity of more than one gene product. The role of the type IIb protein, whether the protein is acting alone or in concert with other bacterial products like the protease, in determining the course of a group A streptococcal infection has not been determined.

The predominant serotype of group A streptococci isolated from both uncomplicated pharyngeal infections and severe invasive disease is serotype M1 (5, 6, 12, 13, 15, 18, 19, 27–29). Examination of recent isolates of this M type for a more invasive phenotype have yielded conflicting results. Studies of the restriction fragment profiles for the *speA* genes of type M1 isolates from uncomplicated pharyngeal infections and for the *speA* genes of M1 isolates from severe invasive infections in the United States demonstrated two distinct profiles, suggesting a clonal basis for the resurgence of severe streptococcal disease (5). However, type M1 isolates from severe invasive infections in Europe produced very low levels of toxin A but high levels of toxin B, suggesting that different clones of type M1 organisms may be responsible for the resurgence of disease in different geographic areas (9, 14).

The results of this study suggest that IgG-binding-protein profiles may provide an additional marker that can be used to identify variations among M1 isolates that might, in concert with other putative virulence markers, help to identify subclones of the serotype M1 family that may in turn correlate with different patterns of infection. While the studies presented in this report provide evidence for two IgG-binding phenotypes of serotype M1 group A streptococci, the reason(s) for these differences at the molecular level has not been identified. The different IgG-binding forms of M1-related proteins may relate to subtle changes in the M1 gene sequence, which have been identified in other studies by sophisticated genetic analysis (11), or to changes in invasive potential in an in vitro tissue culture model (17), or they may relate to some post-translational processing event. Detailed genetic comparison of *emm*(-like) genes from serotype M1 isolates expressing each IgG-binding phenotype will be required to understand the reason for the observed functional differences and to determine if these differences influence the ability of group A streptococci to interact with the host and cause different patterns of disease.

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