thought to affect the probability of quantum release (*22*). Because LTP is triggered postsynaptically (*23*), after its postsynaptic induction a retrograde messenger could be released, affecting presynaptic Ca^{2+} homeostasis and yielding Ca^{2+} oscillations and increased transmitter release. It is possible that different synaptic inputs active during the induction of LTP become associated by showing synchronized, oscillating potentiated transmission.

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10. Because the recording noise variance and quantal variance are additive, finding a variance equal to that of the noise implies that there is negligible quantal variance relative to the noise, which, in this case, has a variance close to the quantal amplitude, ~1 to 2 pA. For comparison, see G. Hess and co-workers [*Neurosci. Lett.* 77, 187 (1987)] and R. Sayer and co-workers [*J. Neurosci.* **10**, 826 (1990)]. Estimates of the number of channels responsible for the quantum depend on cable filtering, single-channel conductance, and open times; none of these values is known for these synapses. The quantal bumps on the density estimate of the amplitude distribution appeared not to be an artifact of a small sample size because they appeared only to the right of the zero peak and because amplitude distributions generated with every other sweep were similar.
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24. These are presumably action potentials mediated by Ca^{2+} channels thought to be located at dendrites [R. Westenbroek, M. Ahlijanian, W. Catterall, *Nature* **347**, 281 (1990)] and may be used as an indicator of dendritic depolarization.

25. I thank R. W. Tsien for support and discussions and A. Jones of Reed College for illuminating statistical help. Supported by University of Iowa College of Medicine Startup Grant to R.M. and a Javits Investigator Award to R.W.T.

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Effect of Light Chain V Region Duplication on IgG Oligomerization and in Vivo Efficacy

WALTER SHUFORD, HOWARD V. RAFF, J. WILLIAM FINLEY, JAMES ESSELSTYN, LINDA J. HARRIS*

A human immunoglobulin G_1 (IgG_1) antibody oligomer was isolated from a transfected myeloma cell line that produced a monoclonal antibody to group B streptococci. Compared to the IgG_1 monomer, the oligomer was significantly more effective at protecting neonatal rats from infection in vivo. The oligomer was also shown to cross the placenta and to be stable in neonatal rats. Immunochemical analysis and complementary DNA sequencing showed that the transfected cell line produced two distinct kappa light chains: a normal light chain (L_n) with a molecular mass of 25 kilodaltons and a 37-kilodalton species (L_{37}), the domain composition of which was variable-variable-constant (V-V-C). Cotransfection of vectors encoding the heavy chain and L_{37} resulted in production of oligomeric IgG.

BOTH IMMUNOGLOBULIN M (IGM) and IgG antibodies can enhance phagocytic clearance of microbial pathogens. Each immunoglobulin class has unique properties, which if appropriately combined could result in an antibody with improved characteristics. Because of their pentameric structure, IgM antibodies can exhibit higher avidity binding to antigen than IgG antibodies with identical intrinsic affinities. Activation of complement component C1 requires multivalent binding of the C1q subunit of C1. Such binding can be provided by a single pentameric IgM molecule or at least two IgG molecules in close proximity (*1*). Therefore, on a per molecule basis, IgM antibodies are often more efficient opsonins. IgG antibodies, however, have the advantages of extended in vivo half-life and, because of their ability to interact with Fc receptors, cross the placenta and contribute to neonatal immunoprophylaxis (*2*). A human IgM monoclonal antibody (MAb) was developed as a potential immunotherapeutic agent for neonatal bacterial sepsis caused by group B streptococci (GBS) (*3*). To compare the in vitro and in vivo activities of IgG_1 and IgM forms of this antibody, we used recombinant DNA techniques to produce both antibody classes. During the production of the IgG_1 antibodies, an oligomeric IgG was formed that appears to have combined some of the desirable characteristics of both IgM and IgG antibodies. We now compare the molecular composition and functional properties of the oligomeric and monomeric IgG antibodies.

The vector pNkA1.1 consists of a complete kappa (κ) light (L) chain gene derived

W. Shuford, H. V. Raff, J. Esselstyn, Department of Immune Sciences, Bristol-Myers Squibb Pharmaceutical Research Institute–Seattle, Seattle, WA 98121. J. W. Finley and L. J. Harris, Department of Molecular Immunology, Bristol-Myers Squibb Pharmaceutical Research Institute–Seattle, Seattle, WA 98121.

*To whom correspondence should be addressed.

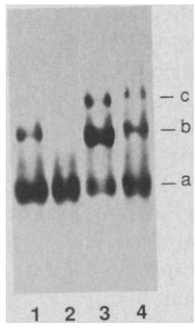

Fig. 1. Urea-PAGE analysis of transfected cell–derived antibodies. MAbs 1B1 and 23B1 were purified by protein A–affinity chromatography. LMM and HMM fractions of MAb 1B1 were obtained after gel filtration on a fast protein liquid chromatography (FPLC) column. Urea-PAGE (3 to 5% acrylamide gradient) was performed with a low pH discontinuous buffer system containing 0.5 M urea (*4, 16*). After electrophoresis, proteins were transferred to nitrocellulose paper and probed with goat antibodies to human γ and κ chains (*3*). Lane 1, unfractionated MAb 1B1 (15% oligomer); lane 2, LMM fraction of MAb 1B1 (99% monomer); lane 3, HMM fraction of MAb 1B1 (85% oligomer); and lane 4, unfractionated MAb 23B1. Bands were designated a, b, and c, as described in text.

from the cell line 4B9 (which makes a human IgM to GBS) (*3*) inserted into pN.1, a derivative of pSV2-neo (*4*). This vector was transfected into the nonimmunoglobulin producing myeloma line P3X63Ag8.653. Supernatants from microtiter plate wells containing G418-resistant cells were screened for cytoplasmic L chain with a fluorescence-based assay (*5*). Cells were cloned in soft agarose (*3, 4*) and a clone that produced relatively large amounts of L chain (B4-1) was selected as the transfection recipient of pNγ1A2.1, a vector carrying the Ig γ_1 heavy (H) chain with the V_H gene segment from the 4B9 cell line (*4*). Transfectants were selected (*6*) and screened for IgG production with an enzyme-linked immunosorbent assay (ELISA). Supernatants containing IgG were further screened for binding to a GBS clinical isolate (*3*). Unexpectedly, one microtiter plate–well culture supernatant (1B1) contained a MAb with specific activity (ELISA absorbance value per microgram of MAb) that was four times that of other supernatants.

When analyzed by gel filtration chromatography (*7*), MAb 1B1 separated into two peaks: approximately 85% eluted as low molecular mass material (LMM) of a size consistent with monomeric IgG (160 to 180 kD), whereas 15% eluted as high molecular mass material (HMM) in the range expected for IgG multimers (>300 kD) (*8*). IgG MAbs from nonrelated transfected myeloma lines eluted as single monomeric peaks. The two MAb 1B1 peak components were characterized by immunochemical techniques. When subjected to conventional, Laemmli, nonreducing, SDS–polyacrylamide gel electrophoresis (PAGE), both HMM and LMM material migrated essentially as monomeric IgG (*8*) (see below). Electrophoresis in the presence of 0.5 M urea and with a low pH discontinuous buffer system (in the absence of SDS) separated the HMM material into three bands (Fig. 1). The mobility of the fastest migrating band (band a) corresponded to that of monomeric IgG (*8*), whereas the mobilities of bands b and c were consistent with those of dimeric and trimeric IgG, respectively. The HMM fraction contained predominantly (~85%) dimeric IgG, with smaller amounts of monomeric and trimeric IgG, whereas the LMM fraction was almost completely monomeric. We concluded that the IgG oligomers were held together by noncovalent interactions that were sensitive to dissociation under some (2% SDS) but not all (0.5 M urea) denaturing conditions.

Unfractionated MAb 1B1 demonstrated an unusual banding pattern when analyzed under reducing and nonreducing SDS-PAGE conditions. Under reducing conditions, MAb 1B1 showed an unexpected band of 37 kD in addition to the typical L and H chains (25- and 50-kD bands, respectively) found in control MAbs (Fig. 2A). After immunoblotting, the 37-kD and 25-kD bands reacted with human κ L chain–specific reagents but not with H chain–specific reagents (*4, 8*). The 37-kD band was designated L_{37}. Nonreducing SDS-PAGE separated MAb 1B1 into three closely spaced bands, with the lowest molecular mass band having the same mobility as normal IgG_1 (Fig. 2B); results from two-dimensional PAGE (first direction, nonreducing conditions; second direction, reducing conditions) were consistent with a normal H_2L_2 IgG stoichiometry for all three bands, but with an L chain composition of either two L_{37}, one L_{37} and one normal L (L_n) chain, or two L_n, for the high (203 kD), medium (190 kD), and low (180 kD) molecular mass bands, respectively (Fig. 2C). Examination of the HMM and LMM 1B1 fractions by nonreducing SDS-PAGE showed the HMM fraction to be enriched in the 203- and 190-kD species and the LMM fraction to have significantly reduced amounts of the 203-kD band (*7*), indicating that L_{37} might be important for oligomer formation.

To investigate the nature of L_{37}, we screened a cDNA library derived from the 1B1 cell line with a probe specific for the 4B9 variable κ chain (V_κ) gene segment. DNA sequencing revealed that one cDNA clone contained a repeat of the V region exon, giving the structure leader-L′V-L′V-C (L′V represents the exon encoding the last three amino acids of the leader sequence and the rearranged variable region; C represents the constant region). This structure was consistent with an amino acid analysis of L_{37} and with Southern blots of genomic DNA from the 1B1 cell line (*9, 10*).

To determine whether expression of L_{37} and H chains would result in oligomer formation, a vector encoding leader-L′V-L′V-C (pGkA1.12) was cotransfected with an H chain vector (*11*) into P3X63Ag8.653 cells. The resulting transfectants produced predominantly the L_{37} chain with trace amounts of L_n (presumably because of alternative RNA splicing). Ten microtiter plate–well culture supernatants most likely to contain oligomer were identified by the highest antigen-binding activity per microgram of IgG. The presence of oligomer was confirmed in all ten supernatants by immunoblotting samples after electrophoresis in the urea-PAGE system. The percentage of oligomerized IgG varied from clone to clone with some transfectants (for example, clone

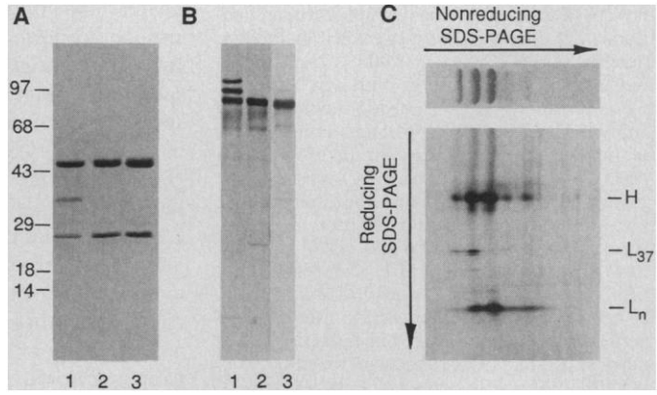

Fig. 2. SDS-PAGE analysis of unfractionated MAb 1B1. (**A**) Reduced protein samples were subjected to electrophoresis through 5 to 15% linear gradient SDS-polyacrylamide gels under reducing conditions. Protein was detected with Coomassie blue R-250 stain (*17*). Lane 1, MAb 1B1; lane 2, monomeric IgG_1 MAb; lane 3, monomeric IgG_2 MAb. The positions of molecular mass markers (in kilodaltons) are shown. (**B**) Conditions and samples were as described in (A), except samples were prepared, and electrophoresis was performed without reducing agent, and proteins were stained with silver nitrate (*18*). (**C**) Two-dimensional gel electrophoresis of unfractionated MAb 1B1. For the first dimension, MAb 1B1 was prepared and subjected to electrophoresis as described in (B). After electrophoresis, the sample lane was excised and boiled for 10 min in sample buffer containing 1% β-mercaptoethanol, and proteins were subjected to electrophoresis under reducing conditions and stained with silver nitrate. The positions of H, L_{37}, and L_n chains are indicated.

23B1 in Fig. 1) producing a greater proportion of oligomer than the original 1B1 cell line. In control experiments, expression of L_n and H chains resulted in expression of only monomeric IgG. In another experiment, we were able to produce oligomeric IgG_2 by transfecting a γ_2 H chain with the 4B9 V region into the B4-1 cell line (*10*). These data indicated that the L_{37} chain was responsible for oligomer production.

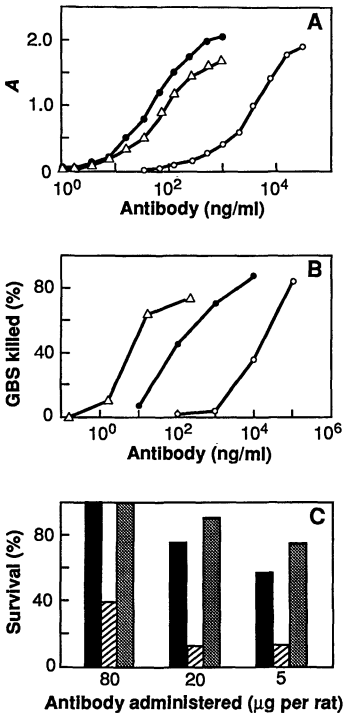

Fig. 3. Functional analyses of monomeric and oligomeric IgG. (**A**) ELISAs comparing LMM (open circles) and HMM fractions (closed circles) of MAb 1B1 and an IgM MAb to GBS (t4B9; open triangles) for reactivity to serotype III GBS strain COH 1 (*19*) immobilized to poly-L-lysine–coated microtiter plates (*3, 20*). Bound antibodies were detected with H chain–specific, peroxidase-conjugated polyvalent antisera (*3, 20*). Because different second-step antibodies were used, IgM and IgG binding activities cannot be directly compared. (**B**) Opsonic phagocytic studies comparing the activity of the same antibody preparations [symbols as in (A)]. Reaction mixtures for opsonic assays consisted of GBS strain IIIR (*21*), human neutrophils, human complement, and antibody (*3*). The data were calculated as follows: percentage GBS killed = 100 × [1 − (CFU remaining after incubation with test antibody/CFU remaining after incubation with control antibody)]; CFU, colony-forming units. Values of duplicate samples were within 4% of each other. When this experiment was repeated, the three MAb preparations had the same relative activities. (**C**) Comparison of the protective activities of LMM (striped bars) and HMM (stippled bars) fractions of MAb 1B1, and t4B9 (solid bars). Experimental and control MAbs (<10 pg of endotoxin per dose) were administered intraperitoneally to 2- to 3-day-old Sprague-Dawley rat pups 2 hours before challenge with 500 CFU [1 LD_{90} (90% lethal dose) of GBS, serotype Ic (*3*)]. Symptoms and survival were scored for 5 days (*22*). When compared at a dose of 20 micrograms per rat, the oligomeric IgG was significantly more effective than the monomer [$P < 0.01$ (n = 20 to 25); Fisher's exact test of categorical data (*23*)]. Values are the sum of three experiments, each showing the same trend.

Three methods for comparing the activity of MAbs to GBS (IgM and MAb 1B1 LMM and HMM fractions) were used: antigen binding, opsonic activity, and animal protection (*3*). In an antigen-based ELISA (Fig. 3A), the HMM fraction appeared similar to the IgM antibody and was 100-fold more active than the LMM fraction (*12*).

Although antibodies must bind to antigen to be opsonic, binding alone does not predict whether antibody will effectively enhance phagocytosis. Therefore, the MAbs were compared in their relative abilities to opsonize a GBS serotype III human clinical isolate. The oligomer fraction was 100-fold more active than the monomer but one-tenth as effective as IgM (Fig. 3B).

A neonatal rat infection model was used to determine whether the enhanced opsonic activity correlated with in vivo protection. Two hours after receiving MAb, newborn rat pups were infected with GBS bacteria. The IgM MAb and the oligomer possessed comparable protective activity at a dose of 5 μg per rat, whereas the monomer was only minimally protective even at a dose of 80 μg per rat (Fig. 3C). Together these functional data suggest that the oligomeric MAb has enhanced binding activity (compared to the IgG monomer), which contributes to the phagocytic clearance of GBS and protection of lethally infected animals.

Transplacental passage and in vivo half-life of the MAbs were also assessed in late gestational stage pregnant rats. Two to 3 days before delivery, timed pregnant females received intravenous administration of IgG monomer (50 μg), IgG oligomer (50 μg), or IgM (200 μg). Beginning shortly after delivery and continuing for 3 weeks, serum antibody concentrations were quantified by IgG- and antigen-specific ELISAs (both specific for human antibodies). The IgG-specific assay detects monomer and oligomer essentially equivalently (*12*). The antigen-binding ELISA is 100 times more sensitive for the oligomer than the monomer and is therefore useful for determining the integrity of the oligomer. In the dams, the monomer and oligomer both had half-lives of ~3 days, whereas in the pups, the monomer and oligomer half-lives were extended to approximately 10 and 7 days, respectively (*13*). In contrast, the IgM half-life was 1 day in the dams, and the antibody was not transplacentally passed to the pups (lower detection limit of 1 ng/ml). Mean oligomer concentrations (± SD; for dams, n = 3; for pups, n = 6) of the sera of dams and pups on the day of delivery were 290 ± 120 and 360 ± 120 ng/ml, respectively. Thus, the oligomers, despite their increased size, still crossed the placenta and, after 3 weeks of circulation, retained the characteristic enhanced antigen-binding activity.

MAb and recombinant DNA technologies allow the selection of antibodies with virtually any specificity, coupled with the capability of changing the antibodies into any human or mouse class (*14*). Attempts have also been made to enhance antibody activity through modifications of affinity and hinge flexibility (*15*). Oligomeric IgG offers an opportunity to improve the avidity of IgG MAbs while retaining Fc region–mediated activities such as complement-dependent opsonization and transplacental passage. Alternatively, if Fc-mediated functions are not required, this approach can be applied to producing $F(ab')_2$ antigen-binding fragment oligomers. Avidity enhancement, such as that observed for the GBS antibody, will most likely vary, depending on the intrinsic affinity of binding and the geometric arrangement of antigenic epitopes. However, spatially proximate epitopes are characteristic of many bacterial, viral, and tumor-associated antigens. In the future, we anticipate determining whether oligomeric IgG antibodies are more active in mediating complement-dependent and antibody-dependent cellular cytoxicity in a tumor model system.

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7. Gel filtration chromatography was performed on Superose 6 (Pharmacia) with phosphate-buffered saline.
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9. V. Smith, unpublished data.
10. L. J. Harris and W. Shuford, unpublished data; the sequence of the 4B9 human κ L chain variable region has been deposited in GenBank, accession number M63438, description 4B9 Vκ15.
11. The pNγ1A2.2 vector is identical to pNγ1A2.1 (*4*) except that it lacks the Bam HI–Hind III fragment containing the γ switch region.
12. The IgG quantitative ELISA yields an ~20% larger signal with purified oligomer than with monomeric antibody with identical H and L chains.
13. The same dams were followed continuously, whereas blood could only be removed from pups once;

therefore, antibody half-lives in pups were calculated with the use of different littermates at each sampling time. Antibody half-lives were calculated from the antigen-binding ELISA data with a computer software program [R. Shumaker, *Drug Metab. Rev.* **17**, 331 (1986)].
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