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Comparative Analysis of Glycosylated and Nonglycosylated Filarial Homologues of the 20-Kilodalton Retinol Binding Protein from *Onchocerca volvulus* (Ov20)

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Ov20 is a structurally novel 20-kDa retinol binding protein secreted by *Onchocerca volvulus*. Immunological and biological investigation of this protein has been hampered by the inability to maintain *O. volvulus* in a laboratory setting. In an effort to find a system more amenable to laboratory investigation, we have cloned, sequenced, and expressed cDNA encoding homologues of Ov20 from two closely related filarial species, *Brugia malayi* (Bm20) and *Acanthocheilonema viteae* (Av20). Sequence comparisons have highlighted differences in glycosylation of the homologues. We present here an analysis of mouse immune responses to Ov20, Bm20, and Av20. The results suggest a strong genetic restriction in response to native Bm20 that is overcome when recombinant, nonnative material is used. Reactivity of human filarial sera to the three recombinant proteins confirmed previous specificity studies with Ov20 but highlighted important differences in the reactivity patterns of the *O. volvulus* and *B. malayi* homologues that may be due to differences in glycosylation patterns. Ov20 is a dominant antigen in infected individuals, while Bm20 is not. The availability of the *B. malayi* homologue enabled us to use defined murine reagents and inbred strains for genetic analysis of responsiveness in a way that is not possible for Ov20. However, the close sequence similarity between Ov20 and Av20 suggests that the *A. viteae* model may be more suited to the investigation of the biological functions of Ov20.

Infection of humans by parasitic filariae leads to a spectrum of clinical manifestations ranging from a remarkable absence of responsiveness to severe immunopathological conditions like elephantiasis and irreversible blindness (17, 21, 22). The final outcome of an infection, whether it be protective immunity, tolerance, or disease development, is largely dependent on the way in which the host immune system recognizes and responds to these multicellular organisms (16, 21, 22). To understand and unravel these complex immunological interactions between parasites and their mammalian hosts, it is crucial to look at specific responses to individual antigens.

Ov20 is an immunodominant glycoprotein antigen of *Onchocerca volvulus*, the causative agent of river blindness in humans. A major part of the clone (OvMBP20/11) has shown a high degree of specificity to onchocerciasis sera, with sera from related nematodes failing to react despite homologues of this protein being present in the other species (3, 4). The protein has been incorporated in a serodiagnostic tool to analyze human immune responses to *Onchocerca* infection (4, 5, 24). Fluorescence-based ligand binding assays show the protein to contain a high-affinity ligand binding site for retinol; algorithms which predict secondary structure indicate a predominance of alpha helices, with no evidence of the beta structures seen in retinol binding proteins characterized so far (15). Ov20, thus represents a new class of helix-rich retinol binding protein of unknown function and appears to be confined to nematodes (15, 31).

The full-length cDNA corresponding to Ov20 has been isolated, and extensive database searches failed to detect similarity to proteins of known function; however, its antigenic and sequence conservation in a wide range of nematodes suggests an important biological role (31). The only other family of retinol binding proteins of similar size and helicity are individual units of the polyprotein allergens of nematodes such as ABA-1 of *Ascaris suum* and gp15/400 of *Brugia malayi* (13, 14).

Retinoids have been implicated in a variety of biological functions in vertebrate and nonvertebrate systems (6, 7, 11, 12, 29). *O. volvulus* is known to sequester retinol to a concentration eight times in excess of the surrounding host tissue (30). Initial clinical manifestations of onchocercal eye damage include night blindness, a symptom consistent with retinol deficiency (25). Although the in vivo function of Ov20 with respect to both the worm and the host is yet to be established, retinol segregation, initial symptoms compatible with retinol deficiency, and secretion of a unique immunodominant retinol binding protein suggest that this molecule is worthy of further investigation. The lack of an animal model for onchocerciasis has hampered further characterization of Ov20, creating a need for homologues of this molecule to be cloned and expressed in parasite systems where animal work is possible. We selected the human filarial parasite *B. malayi*, the causative agent of lymphatic filariasis, and the rodent filarial parasite *Acanthocheilonema viteae*, which because of its close phylogenetic relationship to *O. volvulus* has been considered a model for onchocerciasis.

The objectives of this study were twofold: first, to identify by cloning of the homologous proteins a reliable candidate for modelling the immune response to Ov20 and second, to analyze the immune reactivity of infected mice and humans to the different recombinants to elucidate differences in protein structure that may influence immune responsiveness. Here we report on the isolation and expression of cDNA encoding the homologues of Ov20 in *B. malayi* (Bm20) and *A. viteae* (Av20).

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Sequence comparisons highlighted differences in the pattern of glycosylation in the homologues, allowing evaluation of the role of glycosylation in antigen recognition. Further, the availability of sera from inbred mice inoculated with *B. malayi* allowed a genetic analysis of responsiveness that would not be possible for Ov20.

MATERIALS AND METHODS

Cloning of Av20. DNA encoding the *A. viteae* homologue was amplified from an adult cDNA library with PCR and cloned into pET-15b (Novagen, Madison, Wis.), with an intermediate cloning in pUC18. The oligonucleotide primers for Ov20 used for the PCR (Ov20HTF [5′ CTC CAT ATG GCA AAT GTT GTT CCG TTT TC 3′] and Ov20HTR [5′ CTC GGA TCC TTA ATG TTT TCC GGC ACC 3′]) were designed to generate a protein starting from the predicted cleavage site for the signal peptide, Asn17, of the Ov20 sequence. The inserts which were originally cloned into *Sma*I-digested pUC18 by blunt-end ligation and transformed into the *Escherichia coli* DH5α were subcloned into pET-15b by using the *Nde*I and *Bam*HI sites.

Cloning of Bm20. Oligonucleotides derived from the Ov20 sequence were used to find the *Brugia* homologues. To obtain the full-length *Brugia* sequence, the upstream primer was used with an oligo (dT) primer, and the downstream primer was used with a primer for the spliced leader sequence found on most *B. malayi* transcripts. Template DNA was derived from *Brugia* adult or microfilarial cDNA amplified with the spliced leader and oligo (dT) primers courtesy of Bill Gregory. Sequences were also obtained from conventional microfilarial and adult cDNA libraries provided by Steve Williams, Smith College. Oligonucleotides included XBA-1, *Nde*I, *Pst*I, and *Bam*HI sites for ease of cloning. 5′ oligonucleotides used were Ov20XN (TTTCTAGAACCATGGCAAATGTTGTTCCTG), Ov20PB (CCGGATCCTGCAGTCTCTAATTCTTTTGCAGAA), SL1-f3 (GCTCTAG AGCGGCCGCGGGGGTTTAATTACCCAAGTTGGAG), and dGdT (AAT TGGATCCCCCGGGT). Positive PCR products were cloned into pBluescript (Promega, Madison, Wis.) and sequenced. For expression, a new upstream oligonucleotide that corresponded to the newly determined *Brugia* sequence, BM20XN (GTTTCTAGAACCATGGCAAATGTTATTCCTTTCT), was synthesized. This oligonucleotide was used with dGdT, and the amplified product was cloned into pET-15b (Novagen). The *Nco*I site of pET-15b, which allowed expression of the sequence without the histidine tag, was used. The expressed sequence starts with Met15, two amino acids before the predicted peptide cleavage site.

Expression and purification of Bm20, Av20, and Ov20. Bm20 and Av20 were expressed in the *E. coli* BLD21(DE3) with 1 mM isopropyl 1-β-D-thiogalacto-pyranoside (Sigma, Dorset, United Kingdom) induction, and host cells were lysed by lysozyme treatment and sonication as described by the manufacturer (Novagen). Bm20 was purified by fast protein liquid chromatography using an anion-exchange column (Mono Q HR 5/5; Pharmacia, Uppsala, Sweden). Purification initially used an increasing gradient of sodium chloride, and further purification involved the use of step gradients. Expression of Av20 in the pET-15b expression vector utilized the polyhistidine domain to facilitate purification. Studies on Ov20 have shown that the biochemical properties of the Ov20 bearing the His$_6$ affinity tag and protein in which the tag had been removed with thrombin were indistinguishable (15). In the interest of reducing manipulation, therefore, all assays reported here were carried out with the His$_6$ tag fusion protein. Purification of Av20 was by affinity column chromatography as instructed by the manufacturer (Novagen) for high yield. The purity of the recombinants was verified on 15% polyacrylamide gels. Previously prepared Ov20 was available in *E. coli* BLD21(DE3). Expression and purification have been previously described (15).

Sequencing. Template plasmid DNA for sequencing was prepared according to the protocol for the Wizard Miniprep kit (Promega) or Plasmid Mini-kit (Qiagen). Sequencing was performed with an ABI PRISM terminator cycle sequencing ready reaction kit (Perkin-Elmer) using the vector-specific forward and reverse primers, and analyzed on an ABI PRISM 377 (Perkin-Elmer). Sequences and chromatograms were compared and verified by using SeqEd version 1.0.3; translation, reading frame, and predictions of secondary structure, molecular weight, and pI were performed with MacVector version 4.1.4.

Parasite extracts. Live adult worms were obtained from the peritoneal cavities of *B malayi*-infected jirds (*Meriones unguiculatus*). *B. malayi* antigen was prepared by homogenization of adult worms in phosphate-buffered saline on ice followed by centrifugation at 10,000 × *g* for 20 min. The supernatant was passed through a 0.5-μm-pore-size filter, and the protein concentration was determined by Bradford analysis.

Immunization and implantation sera. Immunization sera were obtained from BALB/c (*H-2^D*), CBA (*H-2^K*), BALB.k (*H-2^K*), and B10.D2 (*H-2^D*) mice immunized with Bm20 or whole *Brugia malayi* antigen in Freund's complete adjuvant (Sigma Immuno-chemicals). Implantation sera were from C57BL/6, BALB/c, CBA, and BALB.k mice implanted intraperitoneally with adult *B. malayi*. Sera were taken 3 to 4 weeks after implantation with six viable adult females and three to six viable adult males. Antiserum to recombinant Ov20 and Ov20/11 was generated by immunization of mice with the recombinant molecules produced in *E. coli* (31). Ov20/11 is the fragment encoded by the major part of the Ov20

FIG. 1. Alignment of the predicted amino acid sequences of the retinol binding proteins Bm20, Ov20, and Av20. N-linked glycosylation sites are underlined, and areas of homology among the three recombinants are indicated (∗). The fragment representing Ov11 is highlighted.

cDNA clone, which was selected as a serodiagnostic tool on the basis of its specificity.

Human sera. Onchocerciasis sera (35 samples) were obtained from confirmed microfilaria-positive patients from an area with a high transmission of onchocerciasis, the Sanega river basin in Cameroon. *Wuchereria bancrofti*-positive sera were obtained from microfilaria-positive individuals living in the Philippines or Sri Lanka (22 samples). The *B. malayi* sera (16 samples) were obtained from Sulawesi, Indonesia. The control sera (8 to 10 samples) were obtained from individuals from areas in Ecuador and Sudan where onchoceriasis is not endemic.

ELISAs. The reactivity of the expressed proteins to mice sera was assessed by an enzyme-linked immunosorbent assay (ELISA) at an antigen concentration of 1 μg/ml and a serum dilution of 1/100. Antibody binding was revealed by using an anti-mouse peroxidase conjugate (Dakinopatts, Copenhagen, Denmark) at a dilution of 1/1,000 and tetramethylbenzidine (microwell) as substrate. The human serum ELISAs had an anti-human immunoglobulin G (clone MO 6014; Oxoid, New York, N.Y.) monoclonal antibody as the secondary antibody and an anti-mouse peroxidase conjugate as the tertiary antibody. The plates were read 15 min after addition of the substrate on an ELISA reader at an optical density of 405 nm.

Statistical analysis. Differences in mean for selected data were analyzed for significance by using a paired or unpaired Student *t* test, as appropriate. Degrees of freedom were calculated accordingly. $P < 0.05$ was taken as significant.

Nucleotide sequence accession number. The fully confirmed Bm20 sequence was submitted to GenBank and given accession no. U69169.

RESULTS

Sequence comparison. Homologues of Ov20 were cloned from *B. malayi* and *A. viteae* and sequenced for comparison with the *Onchocerca* molecule. Expression and purification of the recombinant homologues allowed the immunological analysis detailed below. Sequence comparison of Ov20, Bm20, and Av20 showed Ov20 and Av20 to be very similar, sharing three N-linked glycosylation sites (NX[S/T]) and differing in only nine amino acids (97% similarity) (Fig. 1). The codings for the three glycosylation sites were identical except for the presence of a serine residue at position 46 in Av20 instead of a threonine as in Ov20. The Bm20 sequence was more divergent, with 26 amino acid differences from Ov20 (91% similarity), resulting in the loss of all three glycosylation sites found in Ov20. However, a new N-linked glycosylation site was located near the carboxy terminus. It is unlikely that this site is used, as previous studies
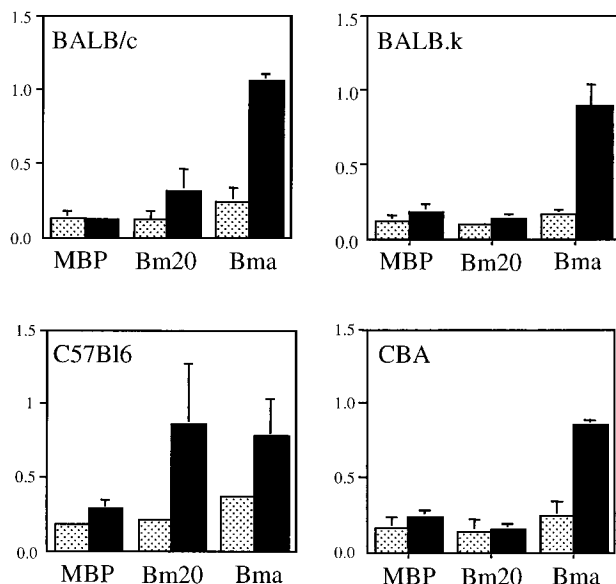
FIG. 2. Reactivity of BALB/c, BALB.k, C57BL/6, and CBA sera from mice implanted intraperitoneally with live adult *B. malayi*. Plates were coated with MBP, Bm20, and *B. malayi* extract (Bma). The *y* axis represents optical density at 405 nm. Dotted bars, control unimplanted animals; solid bars, parasite-exposed animals. Standard deviations represent two to four animals in control groups and three to five animals in implanted group.

have shown no evidence that Bm20 is glycosylated (1a, 31). The signal peptide sequence in Ov20 was conserved in the *B. malayi* homologue. The mature Bm20 and Av20 proteins were predicted to have molecular masses of 18.545 and 18.648 kDa, respectively.

Serological responses in mice exposed to live parasites or recombinant proteins. We have previously done extensive analysis of immune responses in mice implanted with *B. malayi* (1, 19, 20). This provided the necessary reagents for analysis of serum reactivity to Bm20. Sera from mice implanted intraperitoneally with live adult *B. malayi* were assayed for reactivity to parasite antigens Bm20 and whole *B. malayi* antigen, with maltose binding protein (MBP) serving as a nonspecific control. While the BALB/c (H-2^D), CBA (H-2^K), BALB.k (H-2^K) mouse strains failed to show significant reactivity to Bm20, C57BL/6 (H-2^B) mice showed a high reactivity that was significantly different from background MBP (P < 0.01) (Fig. 2). The results suggest a strong genetic restriction in response to native Bm20 as had previously been observed for gp15/400. The genetic restriction observed for gp15/400 was shown to have both *H-2* and non-*H-2* components (1). To assess the role of *H-2* genes in the Bm20 responsiveness, BALB.b (H-2^B) mice were implanted with adult *Brugia* parasites and found to produce antibody to Bm20, suggesting that the response is at least in part *H-2* restricted (data not shown). When the Bm20-reactive C57BL/6 serum was tested against all three recombinant proteins, Av20 and Ov20 were not recognized despite their sequence similarity to Bm20 (Fig. 3). This finding confirms previous specificity studies with Ov20 (4).

Sera from nonresponder mice (B10.D2, BALB.k, BALB/c, and CBA) immunized with *B. malayi* parasite extract in Freund's complete adjuvant also failed to generate an antibody response to Bm20 (data not shown). In contrast, when the studies were repeated with sera from mice immunized with recombinant Bm20, all mouse strains tested (BALB/c, C57BL/6, and B10.D2) exhibited a strong recognition of Bm20 and of Ov20 and Av20 (Fig. 4). Thus, the genetic restriction

and species specificity are overcome when recombinant, nonnative material is used.

Sera from mice immunized with Ov20 had a similar pattern of recognition to the three recombinants; however, sera from mice immunized with Ov11, a truncated version of Ov20 (Fig. 1) that has shown a high degree of specificity for onchocerciasis sera (1), showed a different recognition pattern. The anti-Ov11 sera reacted significantly (P < 0.001) with Av20 and Ov20 in comparison to normal serum controls but responded poorly to Bm20 (Fig. 4).

Serological responses of human filarial sera. In *B. malayi*, a 15-kDa retinol and lipid binding polyprotein allergen (gp15/400) which is immunodominant in elephantiasis has been characterized (1, 14, 23). One of the objectives of this report was to establish whether Bm20 was an immunogen in human filarial infection. The reactivity of the three proteins with a panel of human onchocerciasis and lymphatic filariasis sera was tested. The lymphatic filariasis sera included sera from both *B. malayi*- and *W. bancrofti*-infected individuals. *B. malayi* and *W. bancrofti* are very closely related both phylogenetically and pathologically. They both cause mosquito-borne diseases with identical worm locations in the mammalian host and cause very similar clinical symptoms. Ov20 and Av20 were recognized strongly by onchocerciasis sera (P < 0.001) but not by lymphatic filariasis sera, confirming previous studies on the specificity of Ov20 (Fig. 5) (4). In contrast to Ov20 recognition by onchocerciasis sera, Bm20 recognition by *B. malayi* sera was only marginally significant despite the presence of the homologue in the parasite. *W. bancrofti* sera showed no significant reactivity to any of the three proteins. The slight differences in reactivity between *B. malayi* and *W. bancrofti* is most likely due to differences in geographic localization, as no significant reactivity to Bm20 was observed in 28 *B. malayi* sera representing four clinical categories from Sumatra, Indonesia (data not shown). In all cases, the sera showed strong reactivity to whole parasite extract.

DISCUSSION

Sequence comparison of the Ov20 homologues showed close similarity between Ov20 and Av20 at both nucleotide and amino acid levels, while the Bm20 sequence was more diver-
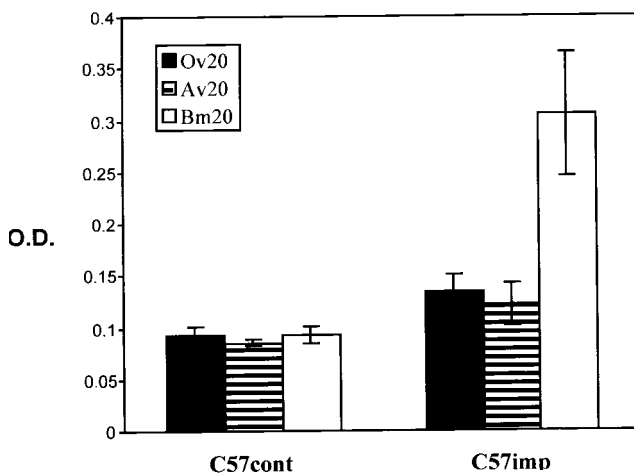

FIG. 3. Reactivity of sera from C57BL/6 mice implanted intraperitoneally with *B. malayi*. Plates were coated with recombinant Bm20, Ov20, and Av20. Standard deviations represent four to six animals in each group. C57cont and C57imp, control and implanted C57BL/6 mouse sera; O.D., optical density.
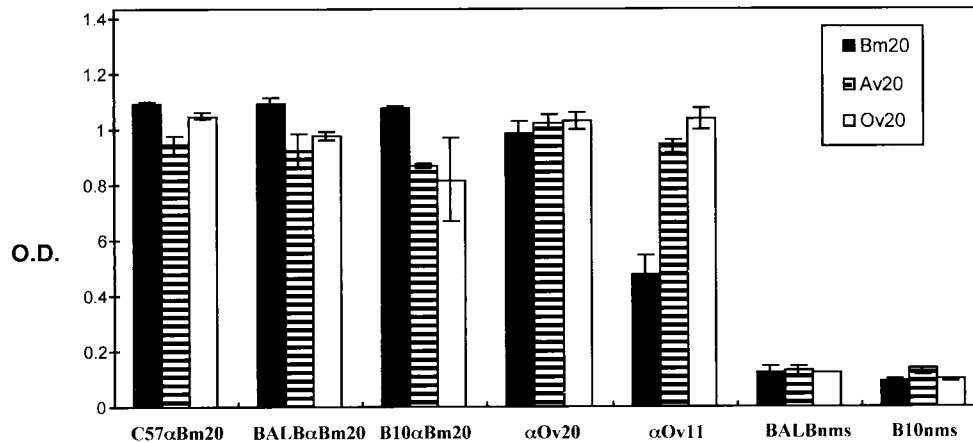
FIG. 4. Reactivity of sera from mice immunized with recombinant Bm20, Ov20, and Ov11. Plates were coated with recombinant Bm20, Av20, and Ov20. Standard deviations represent four to six animals in each group. C57αBm20, BALBαBm20, and B10αBm20, sera from C57BL/6, BALB/c, and B10.D2 mice immunized with Bm20; αOv20, sera from mice immunized with Ov20; αOv11, sera from mice immunized with Ov11; BALBnms and B10nms, normal serum controls from BALB/c and B10.D2 mice; O. D., optical density.

gent. Significantly, major differences were seen in the coding for N-linked glycosylation sites. Three shared sites in Ov20 and Av20 were replaced by a single new site in Bm20. The closer relationship between Ov20 and Av20 compared to Bm20 is not supported by phylogenetic analysis based on small-subunit rRNA sequencing of *O. volvulus*, *B. malayi*, and *A. viteae* (2a), suggesting that the changes may reflect important functional differences between these molecules in the different organisms. The secondary structure predicted for Ov20 is a short alpha helix at the N terminus, corresponding to amino acids 30 to 46 of Ov20, followed by three longer alpha helices (15, 26). Two copies of a degenerate repeat (Ile-Pro-Glu-Glu-tyr/Val-Lys-Asn/Glu-Phe) seen in the region corresponding to the short alpha helix of Ov20 also appears to be conserved in the *B. malayi* and *A. viteae* homologues. The presence of a signal peptide in both Ov20 and Bm20 suggests that the proteins are secreted, but the localization of Bm20 has not been confirmed. Immunoprecipitation studies of the Av20 model have confirmed secretion in all life cycle stages (21a).

In this study, four inbred strains of mice were exposed to Bm20 in three contexts: by infection with live parasites, by adjuvant-assisted immunization with the native molecule, and by immunization with a recombinant molecule. Our results show that the antibody responses to the native molecule are controlled by factors within the H2 complex but that the restriction observed was overcome by immunization with the *E. coli*-derived recombinant protein. We have previously observed a high level of genetic restriction in the murine response to the polyprotein allergen gp15/400 of *B. malayi* at both antibody and T-cell levels (1). These results suggest that features of the native molecule interfere with its ability to be recognized by host T cells.

We had originally proposed that glycosylation alters processing or presentation of the antigen, but the absence of glycosylation in native Bm20 suggests that the restricted response is determined by other factors. It is intriguing to consider that Bm20 and gp15/400, both retinol binding proteins, may bind similar ligands in vivo, which may affect processing or recognition of these molecules. Sera from mice immunized with Ov20 and Bm20 *E. coli* recombinant molecules recognized all three homologues (Ov20, Av20, and Bm20). In contrast, human onchocerciasis sera recognized only Ov20 and Av20, con-
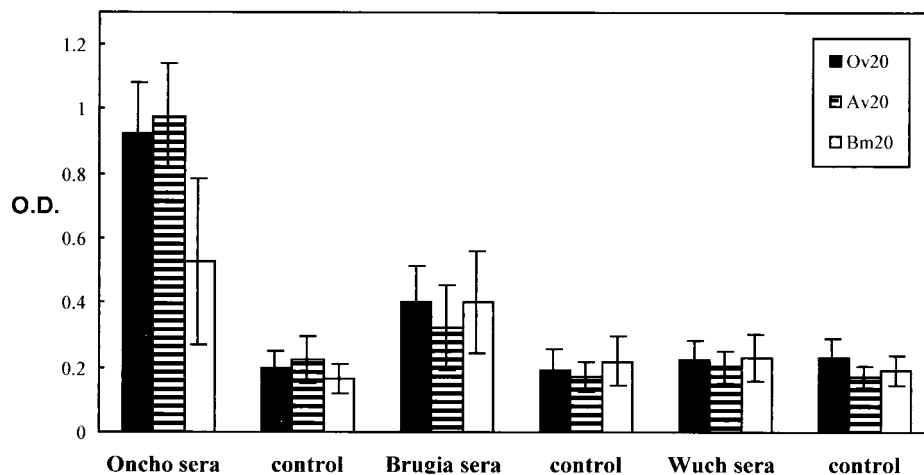

FIG. 5. Reactivity of human filarial (*W. bancrofti* and *B. malayi*) and onchocerciasis sera to the recombinant proteins Bm20, Ov20, and Av20. Standard deviations represent 35 samples in the onchocerciasis (Oncho) serum group, 18 samples in the *Brugia* serum group, 22 samples in the *Wuchereria* (Wuch) serum group and 7 to 8 samples in the control (uninfected) group. O. D., optical density.

sistent with the close sequence relationship between these two proteins. The difference in reactivity to Bm20 observed between mice immunized with Ov20 and its specific fragment Ov20/11 suggests that the central portion of the molecule determines species specificity (Fig. 4). The close relationship between Ov20 and Av20 suggests that Av20 could be used in an *A. viteae* infection model in mice to investigate the biological functions of Ov20, which have up to now been hampered by the inability to maintain *O. volvulus* in a laboratory setting.

The reactivity patterns of Bm20, however, were distinct from those of the other molecules, as sera from patients infected with *B. malayi* failed to recognize it, suggesting that antigenic epitopes are not exposed to the immune system during the natural course of the infection in humans. However, the ability of C57BL/6 to respond strongly to Bm20 during live infection suggests that antigenic epitopes are in fact accessible and that alternative explanations for the lack of reactivity in the human population may be needed. It is possible that active suppression of Bm20 or competition with more dominant antigens or rapid degradation contributes to this nonresponsiveness observed in the human host. Ov20 is immunodominant in *O. volvulus*, but its homologue in *B. malayi* is apparently not. It is unlikely that sequence differences alone could explain the nonresponsiveness of *Brugia* sera to Bm20.

The possible role of glycosylation in immunomodulation is an interesting area of future study. Carbohydrates may be important in determining which epitopes are processed or presented to the immune system (33), and the differential glycosylation observed in the three molecules may contribute to the differential reactivity observed. Furthermore, glycosylation may determine extracellular stability, as one of the key functions of glycosylation is to protect proteins from proteolytic degradation; i.e., Bm20 may be secreted but more rapidly degraded. Yet another possible explanation is the presence of a sheath in *B. malayi* microfilariae which could impede secretion of the protein into host tissue and thereby reduce tissue Bm20 levels significantly. It is intriguing to consider the possibility that gp15/400, a protein which has a profile of retinol binding similar to those of Ov20 (14) and Bm20 (12a) and is both immunodominant and glycosylated, may play the role of Ov20 in *Brugia* infection.

The function of Ov20 (and its homologues) in vivo remains to be established, but there are a number of reasons why this retinol binding molecule may play a critical role in the immunity and pathogenesis of filarial nematode infections. The role of retinoids other than in vision is an area considerable interest (2, 9, 10, 32). Filariae concentrate and store retinol in excess of host tissue (28, 30). Retinoids have been shown to be important in development of a type 2 immune response, with vitamin A-deficient mice displaying a tendency toward a type 1 response, an effect occurring at the level of antigen-presenting cells as well as at the T-cell level (6, 8). Retinoids influence collagen gene expression, and collagen formation plays a major role in the pathogenesis of onchocerciasis (7). It may be relevant here that a 19-kDa retinol binding protein from *O. volvulus* is reported to bind ivermectin, the drug currently used for control of the disease (18, 27, 28). The fact that Ov20 appears to have no counterpart in the mammalian host is encouraging in that it could provide a novel therapeutic target for onchocerciasis therapy.

It is crucial that further studies be done to establish the functional significance of this retinol binding protein, and it is our hope that the cloning of its homologues (Bm20 and Av20) and the availability of two different model systems will help achieve this objective. The greater similarity in both immune reactivity and glycosylation between Av20 and Ov20 suggests

that Av20 would be the better model antigen. In addition, important differences in the reactivity patterns of the *O. volvulus* and *B. malayi* homologues suggest that differences in the immune recognition of this molecule in the two parasite systems may be due to differences in glycosylation.

REFERENCES

1. **Allen, J. E., R. A. Lawrence, and R. M. Maizels.** 1995. Fine specificity of genetically controlled immune response to native and recombinant gp15/400 (polyprotein allergen) of *Brugia malayi*. Infect. Immun. **63:**2892–2898.
1a. **Allen, J. E.** Unpublished data.
2. **Andersen, B., and M. G. Rosenfield.** 1995. New wrinkles in retinoids. Nature **374:**118–119.
2a. **Blaxter, M.** Personal communication.
3. **Bradley, J. E., R. Helm, M. Lahaise, and R. M. Maizels.** 1991. cDNA clones of *Onchocerca volvulus* low molecular weight antigens provide immunologically specific diagnostic probes. Mol. Biochem. Parasitol. **46:**219–227.
4. **Bradley, J. E., K. R. Trenholme, A. Gillespie, R. Guderian, V. Titanji, Y. Hong, and L. McReynolds.** 1993. A sensitive serodiagnostic test for onchocerciasis using a cocktail of recombinant antigens. Am. J. Trop. Med. Hyg. **48:**198–204.
5. **Bradley, J. E., L. Elson, T.I.M., Tree, G. Stewart, R. Guderian, M. Calvopina, W. Parredes, E. Araujo, and T. B. Nutman.** 1995. Resistance to *Onchocerca volvulus*: differential cellular and humoral responses to a recombinant antigen Ov20/11. J. Infect. Dis. **172:**831–837.
6. **Cantorna, M. T., F. E. Nashold, T. Y. Chun, and C. E. Hayes.** 1996. Vitamin A down regulation of IFN-gamma synthesis in cloned mouse Th1 lymphocytes depends on the CD28 costimulatory pathway. J. Immunol. **156:**2674–2670.
7. **Chen, M., S. Goyal, X. Cai, E. A. O'Toole, and D. T. Woodley.** 1997. Modulation of type VII collagen (anchoring fibiril) expression by retinoids in human skin cells. Biochim. Biophys. Acta **1354:**333–340.
8. **Garbe, A. J., J. Buck, and U. Hammerling.** 1992. Retinoids are important cofactors in T cell activation. J. Exp. Med. **176:**109–117.
9. **Giguere, B.** 1994. Retinoic acid receptors and cellular retinoid binding proteins: complex interplay and retinoid signalling. Endocrine Rev. **15:**61–79.
10. **Gudas, L. J., M. B. Sporn, and A. B. Roberts.** 1994. Cellular biology and biochemistry of retinoids, p. 443–520. *In* M. B. Sporn, A. B. Sporn, and D. S. Goodman (ed.), *The retinoids*. Raven Press, New York, N.Y.
11. **Hayashi, A., T. Suzuki, and S. Tajima.** 1994. Modulation of elastin expression and cell proliferation by retinoids in cultured vascular smooth muscle cells. J. Biochem. **117:**132–136.
12. **Hong, W. K., and M. B. Sporn.** 1997. Recent advances in chemoprevention of cancer. Science **278:**1073–1077.
12a. **Kennedy, M. W.** Personal communication.
13. **Kennedy, M. W., A. Brass, A. B. McCruden, N. C. Price, S. M. Kelly, and A. Cooper.** 1995. The ABA-1 allergen of the parasitic nematode *Ascaris suum*. Fatty acid and retinol binding function and structural characterisation. Biochemistry **34:**6700–6710.
14. **Kennedy, M. W., J. E. Allen, A. S. Wright, A. B. McCruden, and A. Cooper.** 1995. The gp15/400 polyprotein antigen of *Brugia malayi* binds fatty acids and retinoids. Mol. Biochem. Parasitol. **71:**41–50.
15. **Kennedy, M. W., L. H. Garside, L. E. Goodrick, L. McDermott, A. Brass, N. C. Price, S. M. Kelly, A. Cooper, and J. E. Bradley.** 1997. The Ov 20 protein on the parasitic nematode *Onchocerca volvulus*: a structurally novel class of small helix rich retinol binding protein. J. Biol. Chem. **272:**29442–29448.
16. **King, C. L., and T. B. Nutman.** 1991. Regulation of the immune response in lymphatic filariasis and onchocerciasis. Immunol. Today **12:**A54–A57.
17. **Kirkwood, B., P. Smith, T. Marshal, and A. Prost.** 1983. Relationships between mortality, visual acuity and microfilarial load in the area of the onchocerciasis control programme. Trans. R. Soc. Trop. Med. Hyg. **76:**862–868.
18. **Lal, P. G., and E. R. James.** 1996. *Onchocerca* retinol- and ivermectin-binding protein activity. Parasitology **112:**211–225.
19. **Lawrence, R. A., J. E. Allen, W. F. Gregory, M. Kopf, and R. M. Maizels.** 1995. Infection of IL-4 deficient mice with the parasitic nematode *Brugia malayi* demonstrates that host resistance is not dependent on a T helper 2 dominated immune response. J. Immunol. **154:**5995–6001.
20. **Lawrence, R. A., J. E. Allen, J. Osborne, and R. M. Maizels.** 1994. Adult and microfilarial stages of the filarial parasite *Brugia malayi* stimulate contrasting

cytokine and Ig isotype responses in BALB/c mice. J. Immunol. **154:**1216–1224.

21. **Maizels, R. M., and R. A. Lawrence.** 1991. Immunological tolerance: the key feature in human filariasis? Parasitol. Today **7:**271–276.

21a.**Nirmalan, N.** Unpublished data.

22. **Ottesen, E. A.** 1992. Infection and disease in lymphatic filariasis: an immunological perspective. Parasitology **104:**S71–S79.

23. **Paxton, W. A., M. Yazdanbakhsh, I. Kurniawan, F. Partono, R. M. Maizels, and M. E. Selkirk.** 1993. Primary structure of immunoglobulin E responses to the repeat subunit of gp15/400 from human lymphatic filarial parasites. Infect. Immun. **61:**2827–2833.

24. **Ramachandran, C. P.** 1993. Improved immunodiagnostic tests to monitor onchocerciasis control programs: a multicenter effort. Parasitol. Today **9:**76–79.

25. **Rodger, F. C.** 1962. A review of recent advances in scientific knowledge of the symptomatology, pathology and pathogenesis of onchocercal infections. Bull. W. H. O. **27:**429–448.

26. **Rost, B., and C. Sander.** 1993. Prediction of protein structure at better than 70% accuracy. J. Mol. Biol. **232:**584–599.

27. **Sani, B. P., A. Vaid, J.C.W. Comley, and J. A. Montgomery.** 1985. Novel retinol binding proteins from filarial parasites. Biochem. J. **232:**577–583.

28. **Sani, B. P., and A. Vaid.** 1988. Specific interaction of Ivermectin with retinol binding protein from filarial parasites. Biochem. J. **249:**929–932.

29. **Semba, R. D., Muhilal, B. J. Ward, D. E. Griffin, A. L. Scott, G. Natadisastra, G. West, and A. Sommer.** 1993. Abnormal T-cell subset proportions in vitamin-A-deficient children. Lancet **341:**5–8.

30. **Sturchler, D. F., F. Wyss, and A. Hanck.** 1981. Retinol, onchocerciasis and *Onchocerca volvulus*. Trans. R. Soc. Trop. Med. Hyg. **75:**617.

31. **Tree, T.I.M., A. J. Gillespie, K. J. Shepley, M. L. Blaxter, R. S. Tuan, and J. E. Bradley.** 1995. Characterisation of an immunodominant glycoprotein antigen of *Onchocerca volvulus* with homologues in other filarial nematodes and *Caenorhabditis elegans*. Mol. Biochem. Parasitol. **69:**185–195.

32. **Wolf, G.** 1996. The regulation of retinoic acid formation. Nutr. Rev. **54(b):**182–184.

33. **Zhiyong, Q. Y., F. Tufaro, and S. Gillam.** 1992. The influence of N-linked glycosylation on the antigenicity and immunogenicity of rubella E1 glycoprotein. Virology **190:**876–881.

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