NOTES

Protein 20, an Immunodominant Surface Antigen of Eimeria bovis†

WILLIAM M. WHITMIRE, JEAN E. KYLE, AND C. A. SPEER*
Veterinary Research Laboratory, Montana State University, Bozeman, Montana 59717

Received 29 July 1988/Accepted 7 October 1988

Autoradiography identified six 125I-labeled proteins, ranging in molecular weight (Mr) from 20,000 to approximately 110,000, on the plasmalemma of Eimeria bovis sporozoites. Immunoblotting with bovine antiserum generated by intravenous inoculations of sporozoites and with immune serum generated by per os inoculations of oocysts revealed that protein 20 (i.e., 20,000 Mr) was an immunodominant antigen on the surface of E. bovis sporozoites.

We are interested in identifying, characterizing, cloning, and expressing the parasite antigens that elicit antibodies and lymphokines with antiparasitic effects against Eimeria bovis, an obligate intracellular protozoan pathogen of the intestinal tract of cattle. Recently, we found that a whole-oocyst antigen preparation of E. bovis would induce the proliferation of bovine T lymphocytes which produce a noninterferon lymphokine capable of inducing bovine and murine macrophages to kill asexual forms of E. bovis and Toxoplasma gondii (3). We have also found that treatment of E. bovis sporozoites with either of two monoclonal antibodies (EbS9 and EbS11) resulted in an approximately 75% decrease in sporozoite penetration of cultured cells (8). Both EbS9 and EbS11 reacted in Western blots (immunoblots) of sporozoites with a protein of 20,000 relative molecular weight (Mr) (called P20).

Since some of the antigens that provide protection against bovine coccidiosis are likely to occur on the surface of sporozoites, we undertook the investigation reported here to determine the relative immunogenicity of various proteins in the plasmalemma of E. bovis sporozoites.

Oocysts of E. bovis were obtained from the feces of experimentally infected Holstein-Friesian bull calves by sucrose flotation, sporulated by aeration in aqueous 2.5% K2Cr2O7, cleaned, and fractured with a motor-driven Teflon-coated tissue grinder (1). Sporozoites were excysted from sporocysts, washed in calcium- and magnesium-deficient Hanks balanced salt solution (pH 7.4), and purified by passage through a nylon wool column (5, 7).

Sporozoite surface proteins were radiiodinated by using lactoperoxidase (6), washed twice in cold phosphate-buffered saline–cysteine–HCl and once in cold phosphate-buffered saline, and solubilized at room temperature in sodium dodecyl sulfate (SDS) solubilizing solution (2% SDS, 10% glycerol, 6.25 \times 10^{-2} \text{ M} \text{ Tris hydrochloride buffer [pH 6.8]}) at a ratio of 6 \times 10^9 sporozoites to 10 \mu l of solubilizing solution. The solubilized sporozoites and prestained molecular size standards (200, 97.4, 68, 43, 25.7, 18.4, and 14.3 kilodaltons; Bethesda Research Laboratories, Bethesda, Md.) were subjected to SDS-polyacrylamide gel electrophoresis in a 12.5% polyacrylamide slab gel by using a discontinuous buffer system (4). The gel was then fixed, dried, and autoradiographed with Kodak X-Omat AR X-ray film (Eastman Kodak Co., Rochester, N.Y.) for 1 to 14 days at −70°C.

Antiserum (AS) was obtained from the blood of a calf 1 week after the second of two intravenous inoculations of 2 \times 10^7 live E. bovis sporozoites in Hanks balanced salt solution that were given at a 6-week interval. Immune serum (IS) was obtained from another calf that had been inoculated per os 1, 5, 9, and 13 weeks earlier with 1 \times 10^5, 5 \times 10^5, 4 \times 10^3, and 4 \times 10^6 oocysts of E. bovis, respectively. Normal serum (NS) was obtained from a coccidium-free calf that was less than 1 month old. All serum samples were stored at −20°C until needed. The immunofluorescence assay titers for im-

* Corresponding author.
† Contribution J-2227 from the Montana Agricultural Experiment Station.

FIG. 1. Autoradiographic profile of 125I-labeled surface proteins of E. bovis sporozoites. The numbers indicate the relative positions of six distinct 125I-labeled protein bands, one of which corresponds to P20 with a Mr of 20,000. Molecular weight standards (10^3) are indicated at the right.
munoglobulin G against *E. bovis* sporozoites in AS, IS, and NS were 1,280, 160, and 0, respectively.

Other sporozoites that were not radiiodinated were solubilized, subjected to SDS-polyacrylamide gel electrophoresis (along with prestained molecular weight standards) in a 12.5% polyacrylamide slab gel, and fixed as described above. The electrophoretically separated sporozoite proteins and molecular weight standards were then transferred (70 V for 4.5 h) from the gel to nitrocellulose paper (Trans-Blot; Bio-Rad Laboratories, Richmond, Calif.) (8). The nitrocellulose paper was fixed, dried, blocked with bovine lacto transfer blocking solution (BLOTTO), cut into 4-mm-wide strips, and probed with AS, IS, or NS (diluted 1:20, 1:3, and 1:3, respectively, in BLOTTO) in a humid chamber overnight at 4°C. Immunospecific binding of AS and IS immunoglobulins was detected by treating the probed nitrocellulose strips with a 1:200 dilution of horseradish peroxidase-conjugated rabbit antiovine immunoglobulin G (heavy- and light-chain specific; Organon Teknika, Malvern, Pa.) in BLOTTO and a peroxidase substrate solution (0.3% 4-chloro-1-naphthol and 16.6% methanol with 5 µl of 30% H₂O₂ in 0.05 M Tris hydrochloride-0.2 M NaCl; pH 7.4) for 1 h at room temperature (2).

 Autoradiographic analysis revealed the presence of six distinct protein antigens (designated P1 through P5, and P20) on the surface of *E. bovis* sporozoites (Fig. 1), five of which had the following *M*ₘ values: P2, 54,900; P3, 42,700; P4, 40,700; P5, 25,000; P20, 20,000. The *M*ₘ of P1 was approximately 110,000, but this could not be determined from a standard curve because the linear relationship of the prestained molecular weight standards was maintained only for *M*ₘ of 14,300 to 68,000 (data not shown).

AS and IS reacted in Western blots against several surface and internal sporozoite antigens, whereas no reaction occurred with NS (Fig. 2). Both AS and IS reacted intensely with the surface protein P20. AS reacted with four of the other five iodinated surface proteins (P2, P3, P4, and P5) with various intensities; IS reacted weakly with three iodinated surface proteins (P2, P3, and P4) (Fig. 1 and 2). An antigen with a *M*ₘ greater than that of P2 (Fig. 2) also reacted intensely with AS and IS in Western blots (Fig. 2). This high-*M*ₘ antigen may correspond to P1, but this could not be determined with certainty because of the lack of linearity displayed by the molecular weight standards in conjunction with the autoradiograph and Western blots in the high-*M*ₘ region.

Western blots with AS and IS and solubilized *E. bovis* sporozoites showed that P2, P3, P4, and P20 were antigenic regardless of the route of immunization. In contrast, P5 reacted with AS that was raised by intravenous inoculation of sporozoites but not with IS obtained from a calf that was inoculated per os and challenged repeatedly with live *E. bovis* oocysts.

The strong reactivity of AS and IS with P20 compared with the relatively low reactivities of P2, P3, and P4 or the nonreactivity of P5 with IS indicates that P20 is an immunodominant sporozoite surface antigen. This is emphasized by the fact that certain proteins which labeled more intensely with ¹²⁵I (such as protein 5) than P20 had less reaction on the Western blots than did P20. Thus, P20 appears particularly promising as a vaccine component because these data indicate that it is an immunodominant surface antigen and our previous work shows that monoclonal immunoglobulin G antibodies that react against it inhibit penetration of cultured cells by *E. bovis* sporozoites (9).

This work was supported by grants 85-CRSR-2-2688 and 87-CRSR-2-3148 from the U.S. Department of Agriculture.

LITERATURE CITED


