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Immunochemical Analysis of Lyme Disease Spirochetes

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Sera from patients with Lyme disease (LD) originating in the United States and Europe were examined by western blot analysis for antibodies to LD spirochete components. Whereas some components reacted with antibodies from the majority of patients, other components, notably an abundant cellular protein with an apparent molecular weight of 34,000 were less commonly bound. This latter proteinaceous component was in a region of the polyacrylamide gel electrophoresis profile which demonstrated variability between different LD spirochete isolates. Thus, there may be more than one serotype of LD spirochete, and the 34,000-range proteins may be a basis for such a distinction.

INTRODUCTION

The etiological agent of erythema chronicum migrans has been isolated and successfully propagated in the laboratory [1-5]. Studies completed or under way will tell us much about the metabolic requirements of the Lyme disease spirochete, its ultrastructure, its susceptibility to antimicrobial agents, and its genetic relatedness to other spirochetes. Nevertheless, we will probably still have much to learn of how this previously unknown spirochete causes the skin lesion, the meningoencephalitis, the cardiac abnormalities [6], and, perhaps most difficult to understand, the arthritis of Lyme disease [7]. Although animal models of infection with this spirochete will be of value in revealing the pathogenesis of Lyme disease, studies of the immune response during human Lyme disease will provide complementary information.

METHODS

Techniques adapted from those of present-day basic biological research permit figurative dissection of the Lyme disease spirochete into components and identification of which components patients form antibody against. One of these techniques is the "western blot" (Fig. 1 schematically shows the western blot procedure). Cellular proteins and some non-proteinaceous components are first separated, according to their molecular weights, by sodium dodecyl sulfate-polyacrylamide gel electrophoresis [8]. The one-dimensionally separated components are then transferred electrophoretically from the gel to nitrocellulose, yielding a replica of the gel pattern on the nitrocellulose membrane matrix [9]. The unoccupied protein-binding sites on the membrane are then "blocked" with an excess of a globular protein such as bovine serum albumin. At this point, the "blot" is incubated with diluted antiserum or hybridoma culture supernatants, washed to remove unbound antibody, and then

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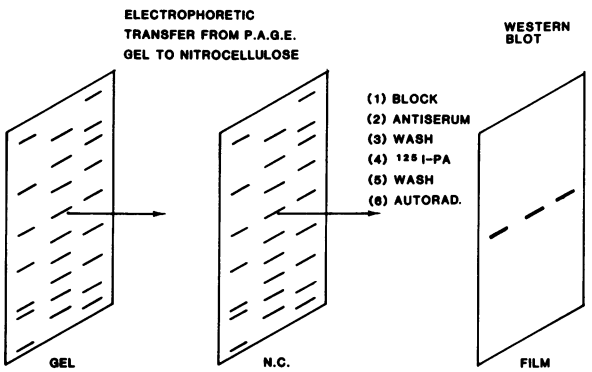

FIG. 1. Western blot procedure. Three hypothetical cell lysates are separated into their constituent components by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (PAGE). The components are then electrophoretically transferred to a nitrocellulose (**N.C.**) membrane. The N.C. is incubated with bovine serum albumin in buffer to block remaining protein-binding sites. Antiserum or monoclonal antibody is reacted with the blot. After being washed, the blot is probed with ^{125}I-labeled protein A (^{125}I-PA) for bound immunoglobulin G. Following a final wash, the N.C. is dried and exposed to film for autoradiography (autorad).

probed for bound antibody with a reagent such as radioiodinated protein A. The modifications of the western blot procedure used in the present studies have been described elsewhere [10,11].

RESULTS

An early application of the western blot to the study of Lyme disease is shown in Fig. 2. The patient, a resident of Shelter Island, NY (patient J.G. of [1]), had erythema chronicum migrans, fever, myalgias, and malaise in June 1979. Blood was drawn and the serum was saved. In April 1980, a convalescent specimen was obtained and along with the acute illness serum was examined by western blot. Except for the dark bands seen in both acute and convalescent sera blots at the top in area of the stacking gel, only the convalescent serum showed evidence of having IgG antibodies that bound to spirochete components.

An expanded western blot study was then undertaken at Rocky Mountain Laboratories in collaboration with Drs. Allen Steere and Edgar Grunwaldt and with the help of several other investigators [11]. The results of this study showed that: *(1)* There are several *Ixodes dammini* or Lyme disease spirochete components to which Lyme disease patients formed IgG antibody. *(2)* Sera from controls, including patients with clinically similar diseases such as rheumatoid arthritis, showed little reactivity with these components. *(3)* There were some cross-reactivities between Lyme disease spirochetes and other spirochetes demonstrated by western blots of Lyme disease sera versus representatives of borrelia, treponemes, and leptospires and by blots using sera obtained from patients with other spirochetal diseases such as secondary syphilis and relapsing fever.

One of the patients in this larger study was a woman who acquired Lyme disease in Rhode Island and went on to develop chronic arthritis. The titer of antibodies in both serum and synovial fluid to strain B31 of the *I. dammini* spirochetes was 1:2,560 measured by indirect immunofluorescence [11]. The synovial fluid was examined by western blot for IgG antibodies to components of not only strain B31, a cloned and many-passaged laboratory strain [2], but also to the original Shelter Island tick isolate (IDS; [1]), from which B31 had been derived and which had been passaged only three times before harvest for the studies, and to strain HB19 [12] isolated from the blood of a patient with Lyme disease [3]. The western blot patterns

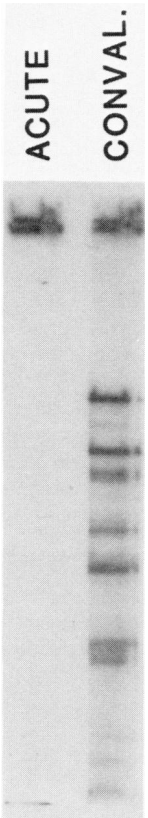

FIG. 2. Western blot analysis. Acute and convalescent **(conval.)** sera from a resident of Shelter Island, NY, with Lyme disease (see text) was reacted with a whole cell lysate of the original *Ixodes dammini* spirochete (IDS) isolate from Shelter Island [1].

of this synovial fluid were, with the exception of differences in reactivities with a very high molecular weight component and some low molecular weight components, identical for the three isolates (Fig. 3). The pattern obtained with serum from this patient was the same as that seen with synovial fluid (data not shown).

Notable in these blots are the reactions of antibodies not only with the ten components previously identified [11] (and now denoted by their apparent molecular weights) but also with an eleventh component with apparent molecular weight of 34 $\times$ 10^3 (34K). This was the same apparent molecular weight of an abundant protein identified in the Coomassie brilliant blue-stained gel. The 34K molecular weight protein was in the region of the gel which has demonstrated variability between different strains [12]. A closer look at this part of the gel is shown in Fig. 4.

When the western blot patterns produced with sera from many Lyme disease patients were examined, however, I found that few sera had detectable antibodies to components in the 34K region (Table 1). The sera used in this accounting were those reported in [11] and samples from additional patients in the United States or Europe. Each serum was drawn late in the course of or during convalescence from an illness that by clinical criteria [13] was definite or probable Lyme disease. All sera had indirect immunofluorescence titers aganist Lyme disease spirochetes of 1 to 80 or greater. In our laboratory, titers of 1 to 80 or greater were seldom found in sera from controls [1,11; Barbour AG: unpublished observations].

When the sera were grouped with regard to continent of origin, six of 34 United

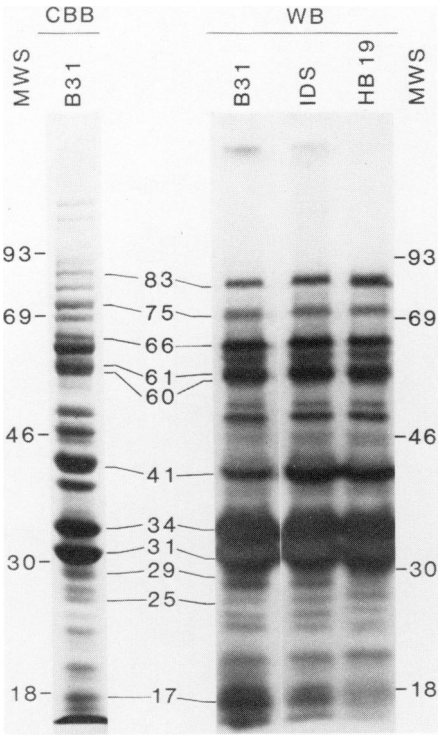

FIG. 3. Western blot analysis of reactivities of synovial fluid from patient with chronic Lyme arthritis (see text). Whole cell lysates of strains B31, IDS, and HB19 were used. The relative mobilities of selected components in the blots as well as in a Coomassie brilliant blue (**CBB**)-stained gel of strain B31 were determined by comparison with the migrations of ^{14}C-labeled molecular weight standards (**MWS**). The MWS (with apparent molecular weights given in daltons × 10^3) were phosphorylase B (**93**), bovine serum albumin (**69**), ovalbumin (**46**), carbonic anhydrase (**30**), and beta-lactoglobulin (**18**). The apparent molecular weights of 11 components in the blots and in the gel are shown. Differences between blots are seen near the top of the gel and in some components with apparent molecular weights below 30 × 10^3.

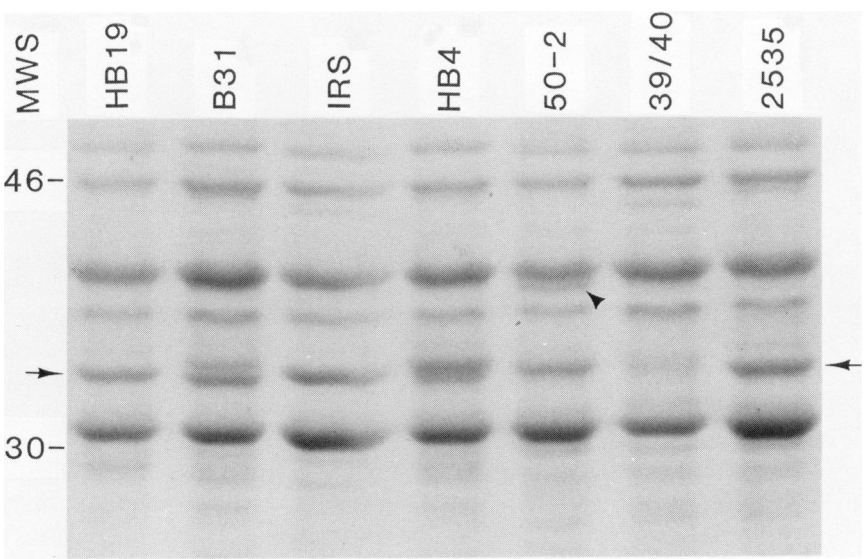

FIG. 4. Coomassie brilliant blue-stained proteins of seven Lyme disease spirochete isolates examined by PAGE. The region between the molecular standards (**MWS**) of ovalbumin (**46**) and carbonic anhydrase (**30**) is shown in detail. The origin of the seven isolates was described in [12]. Large arrows indicate the region in the gel (around 34 × 10^3 daltons) where variation between strains has been noted. Strain B31 in this gel was an earlier passage of the same strain shown in Fig. 3. The closed triangle indicates a protein in strain 50-2 that was not detected in the other isolates.

TABLE 1
Presence in Western Blots of Detectable Bands that Co-Migrated with Lyme Disease Spirochete B31 Components

Apparent Molecular Weight of Component ($\times 10^3$)	Source of Patient Sera[a]	
	United States (N = 34) Number Positive (%)	Europe (N = 9) Number Positive (%)
17	20 (59)	1 (11)
25	15 (44)	1 (11)
29	22 (65)	3 (33)
31	13 (38)	1 (11)
34	6 (18)	0 (0)
41	34 (100)	7 (78)
60	33 (97)	9 (100)
61	27 (79)	4 (44)
66	31 (91)	1 (11)
75	25 (74)	4 (44)
83	30 (88)	3 (33)

[a]Patients with definite or probable Lyme disease and with serum indirect immunofluorescence titers against strain B31 of 1 to 80 or greater

States patients and none of the nine European patients were seen to have detectable antibody to a 34K (or thereabouts) component. In contrast, components with apparent molecular weights of 41K and 60K elicited antibodies from the majority of patients from both the United States and Europe. The remaining components were recognized by antibodies in a higher percentage of United States sera than in European sera.

DISCUSSION

A possible explanation for these results is that the 34K-range proteins are only weakly immunogenic. Alternatively, if they are strongly immunogenic in their native states, perhaps the immunodominant epitopes of the proteins are irreversibly altered during the western blot procedures such that the majority of antibodies no longer recognize them.

Although the western blot studies of serially obtained sera from several patients with Lyme disease have clearly shown that there are individual differences in antibody responses to common components in the Lyme disease spirochetes [11], the finding that some patients, as well as rabbits infected with the spirochetes [1,11], possess antibodies to a 34K component indicates that this component or components are at least potentially immunogenic and that their epitopes are still intact after the western blot procedures.

Thus, a more likely explanation, given the demonstrated variability between strains in this region of the polyacrylamide gel profiles, for the infrequency of sera showing reactivity to the 34K component is that there are different serotypes among these spirochetes. A corollary of this conclusion is that the 34K proteins can perhaps be used to distinguish between serotypes. Indeed, a monoclonal antibody that binds in western blots to the 34K protein of strain B31 reacts in immunofluorescence assays with this and other strains but not with several others [Barbour AG, Tessier SL: Monoclonal antibodies in Lyme disease spirochetes. 1983. Twenty-third Inter-

science Conference on Antimicrobial Agents and Chemotherapy. Abstract 239. Submitted for publication].

The failure to find evidence in European sera of any patients with antibodies to a 34K region component or of more than a few patients with antibodies to some other components suggests that there may be differences in the distributions of various serotypes between Europe and North America. Whether an inhomogenous geographic distribution of serotypes in any way results in dissimilarities between ixodid tick-associated syndromes on each side of the Atlantic, for example, in the apparent lower frequency of arthritis in Europe [6,14], remains to be determined.

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