The Rabbit as a Model for the Study of Lyme Disease Pathogenesis and Immunity—A Review

James N. Miller, PhD; Denise M. Foley, PhD; Jon T. Skare, PhD; Cheryl I. Champion, PhD; Ellen S. Shang, BA; David R. Blanco, PhD; and Michael A. Lovett, MD, PhD

ABSTRACT

Culture-positive erythema migrans (EM) lesions have been induced consistently and reproducibly on the clipped backs of adult, New Zealand white rabbits following the intradermal injection of *Borrelia burgdorferi*. Studies utilizing the B31 strain have shown that EM can be produced after as many as 47 in vitro passages, although the degree of induction is less consistent than with lower passaged organisms. Infiltrating lymphocytes, plasma cells, and macrophages with perivascular cuffing, characteristic of the human EM lesion, were observed within the superficial and deep layers of the EM lesion. Skin infection and dissemination to the popliteal lymph nodes, joint tissue, and spinal cord were also produced and persisted for 8 to 10 weeks after infection. Ultimate clearance of EM and local and disseminated infection less than 3 months after intradermal infection with the development of complete immunity to reinfection are striking features that distinguish the rabbit model from both the monkey and other small animal models. In studies utilizing the B31 strain, the extent of protective immunity was directly related to the extent of prior in vitro passage; passage 4 organisms induced complete protection, while passage 27 and 47 organisms induced corresponding lesser degrees of acquired resistance. Serum from a rabbit completely immune to challenge with the low passage B31 *B burgdorferi* and adsorbed with an avirulent ATCC B31 strain isolate, was used to identify outer membrane (OM)-spanning protein antigens specific for the virulent strain. On the basis of 2-D gel electrophoresis and immunoblot analysis of TX-114 detergent phase OM proteins, seven antigens, including OspD, were found only in passage 10 organisms, two were common to both passage 10 and passage 48 organisms, and one was found only in passage 48 organisms; the acronym, Oms^{vsa}, was used to designate OM-spanning proteins that are only virulent-strain associated.

Immunoelectron microscopy studies using the adsorbed serum paralleled the immunoblot analysis and provided compelling evidence for the presence of antibodies directed against virulent strain associated antigens with putative virulence and protective immunogenic properties. Ongoing and future studies in the rabbit relevant to the understanding of Lyme disease pathogenesis and immunology include: the elucidation of pathogenetic and immune mechanisms that mediate the course of the disease process, clearance of the infection, and the establishment and maintenance of both endogenous and exogenous acquired resistance; evaluation of vaccinogens; and comparison of the rabbit response to needle vs infected tick inoculation.

Key words: Lyme disease, rabbit model, pathogenesis, immunity

INTRODUCTION

Lyme disease, the most common vector-borne disease in the United States,[1] is characterized by the appearance of distinctive erythema migrans (EM) skin lesions in 60% to 85% of patients.[2] Early and late clinical manifestations reflecting dissemination to visceral targets include arthritis, neurological manifestations, lymphadenopathy, and carditis.[2-8] Implicit in the development of control measures for this disease is a complete understanding of the cellular and molecular mechanisms of pathogenesis and immunology operative during the course of the disease. Of imminent importance is the elucidation of those mechanisms at play during and immediately following initial contact between the spirochete and host leading

From the Department of Microbiology and Immunology (Drs Miller, Foley, Skare, Champion, Blanco, and Lovett, and Ms Shang); and Division of Infectious Diseases (Dr Lovett), UCLA School of Medicine, Los Angeles.

Address correspondence to James N. Miller, PhD, Department of Microbiology and Immunology, CHS 43-239, UCLA School of Medicine, Los Angeles, CA 90024.

to the development of the EM lesion(s), dissemination, and latency vs reactivation as opposed to complete and lasting immunity. Animal models in which these parameters can be simulated provides the opportunity to explore essential pathogenetic and immune mechanisms responsible for the entire spectrum of infection and disease as well as those factors that influence the development of acquired resistance.

RODENT AND RHESUS MONKEY MODELS: ADVANTAGES AND LIMITATIONS

In contrast to the rhesus monkey,[9] the small and practical rodents used in experimental investigations lack the ability to produce EM lesions.[10-18] This, together with the chronic persistence of disseminated infection in each of these animal models,[10-19] precludes their use in attempting to elucidate the mechanisms underlying the development of infection-derived acquired resistance against both EM and dissemination.

The characteristic perivascular lymphocytic, plasma cell, and macrophage infiltration observed in human EM lesions and infected viscera[20] has been observed in the EM lesions of the rhesus monkey[9] and in the target organs of dissemination in both the monkey and rodent models.[13-15,18,19,21] However, the role of immune cells during the course of EM development and healing, and during persistence and disappearance of spirochetes from the skin, has not been addressed. Indeed, the continued persistence of spirochetes in rodent and monkey visceral tissues precludes the conduct of studies in these animals designed to determine the immune mechanisms that mediate healing, clearance, and the development of acquired resistance following infection.

Rodents have provided seemingly satisfactory models for evaluating the efficacy of Osp components, *Borrelia burgdorferi* bacterin, and a *B burgdorferi* mutant lacking OspA and OspB as vaccines.[22-29] Passive protection and correlative complement-dependent borreliacidal assays appear to provide compelling evidence for the major role of humoral immune mechanisms operative in protective immunity against disseminated infection in rodents following vaccination or infection.[23,30,31] Unfortunately, the use of "tests of challenge" not capable of evaluating protection against the development of EM lesions places a severe restriction on the ability to interpret accurately both the efficacy of the potential vaccinogen and operative cellular *and* humoral immune mechanisms responsible for acquired resistance.

PREVIOUS ATTEMPTS TO DEVELOP A RABBIT MODEL

Prior to the studies conducted in our laboratory, attempts to induce EM reproducibly in the rabbit were unsuccessful. Although erythematous lesions had been observed, *B burgdorferi* could not be cultured from putative EM, nor could the characteristic histopathology of EM be demonstrated. Burgdorfer and his colleagues described the appearance of annular, erythematous lesions in New Zealand white rabbits after being fed upon by *Ixodes scapularis* infected with *B burgdorferi*.[32] These lesions, however, rarely appeared at the site of tick feeding and did not develop until 4 to 12 weeks after tick engorgement.

Kornblatt and his coworkers attempted to produce EM on the clipped backs of New Zealand white rabbits using three methods: by feeding presumptively infected *I scapularis*, by injecting infected tick organ homogenates, or by injecting a culture of *B burgdorferi* strain G39/40, passage 50.[33] Of the 33 rabbits inoculated by these various methods, one of four rabbits fed upon by infected ticks developed an erythematous skin lesion at the site of tick attachment that persisted for 3 days; however, silver stains of the lesion biopsies showed spirochetes but cultures were negative. Of the 10 rabbits injected intradermally with tick organ homogenates, one developed an erythematous, indurated lesion at two of the three inoculated sites; these lesions, which appeared in 2 days and persisted for 6 days, were silver stain and culture negative. None of the 19 rabbits inoculated both intradermally and intravenously with either tick organ homogenates or the G39/40 strain developed EM.

DISTINCTIVE FEATURES RELATING TO PATHOGENESIS AND IMMUNOLOGY IN THE RABBIT

In recently published studies, we have described the consistent and reproducible induction of culture-positive EM lesions on the clipped backs of adult, New Zealand white rabbits following the intradermal injection of six different low-passaged virulent strains of *B burgdorferi* with concentrations ranging from 10^3 to 10^7 organisms per site[34] (Tables 1 and 2, Fig 1). Studies using the B31 strain have shown that EM can be produced after as many as 47 in vitro passages, although the degree of lesion induction is less consistent than with lower passaged organisms (Table 1). EM lesions at the injection site appeared within 5 days after infection and persisted an average of 7 days. Infiltrating lymphocytes, plasma cells, and macrophages with perivascular cuffing, characteristic of the human EM lesion, were observed within the superficial and deep dermal layers of the EM lesion.[34] Skin infection and dissemination to the popliteal lymph nodes, joint tissue, and spinal cord also were produced[34] (Tables 3-A, 3-B, and 4).

Further studies demonstrated that one strain of *B burgdorferi* could be cultured from the skin, joint tissue,

Table 1
EM After ID Inoculation of Rabbits With B burgdorferi *B31 Strain*

	Inoculum*				
	10^7	10^6	10^5	10^4	10^3
	EM/Total sites				
In vitro passage					
4	8/8	8/8	8/8	ND	ND
8	8/8	8/8	8/8	8/8	8/8
27	8/8	8/8	8/8	8/8	8/8
47	8/8	8/8	4/8	8/8	6/8
ATCC B31					
Avirulent control	0/8	ND	ND	ND	ND

**Two rabbits were injected at four sites for each inoculum tested.*
ND, not done.
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Table 2
Production of EM After ID Inoculation of Rabbits With Seven Strains of B burgdorferi

		Lesions	
Strain*	EM/sites	Time of appearance	Duration
		d‡	*d*§
B31	24/24	3-8	1-9
N40	24/24	4-5	5-19
SH-2-82	24/24	4-7	5-15
HB19	24/24	4-7	2-7
297	24/24	4-5	1-6
Ca-2-87	24/24	4-6	5-9
ECM-NY-86	11/24	5	5-7
Heat-killed	0/8	N/A	N/A
ATCC Avirulent B31	0/8	N/A	N/A

**Four rabbits were inoculated per strain with 10^7 organisms per site. Controls include two animals inoculated with 2 x 10^7 heat-killed organisms (56°C for 45 min) from each strain and two animals that were inoculated with 4 x 10^7 ATCC B31. Bladder isolates from infected mice were used at in vitro passage 1. ‡Mean = 5±1; §mean = 7±3. N/A, not applicable.*
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and spinal cord; one strain from skin and joint tissue; two strains from skin and spinal cord; and two from the skin only (Table 4). Although preliminary, these findings are in accord with the possibility that certain strains of *B burgdorferi* may have a specific affinity for the skin, central nervous sysem, and/or joints. Organisms persisted in the skin and viscera for 8 to 10 weeks after infection[34] (Table 3-B, Fig 2, unpublished data).

This ultimate clearance of EM as well as local and disseminated infection within 3 months of intradermal infection is a striking feature that distinguishes the rabbit model from both the monkey and other small animal models. Resolution of the infection provides a convenient means for studying the ontogeny of those immune mechanisms that may contribute to EM development and those responsible for clearing the infection. In preliminary studies, we have shown that within 12 hours of the intradermal inoculation of normal and immune rabbits with 10^7 B31 passage 4 *B burgdorferi*, an infiltration of polymorphonuclear leucocytes appears at the site of inoculation in both the naive and immune animals and persists for up to 4 days.[35]

In the immune animals in which EM lesions failed to develop during a 28-day observation period, no further

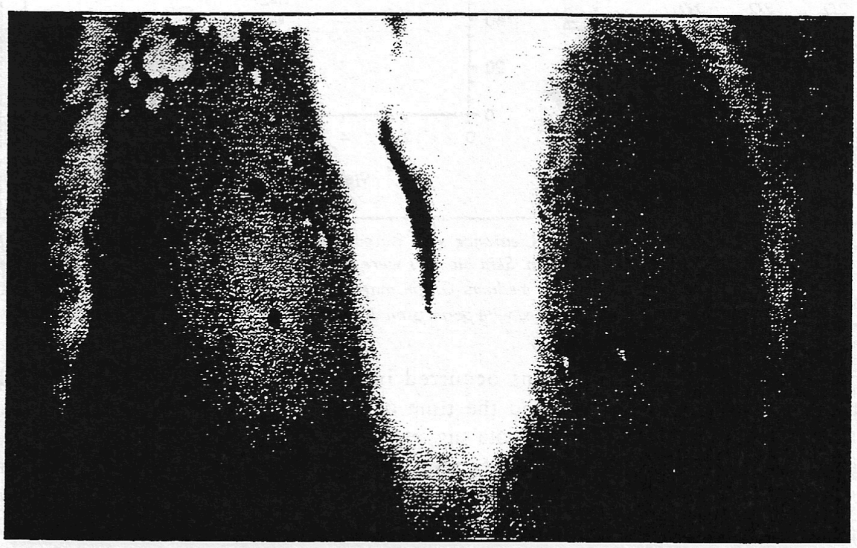

Fig 1. Erythema migrans in the New Zealand white rabbit. Right—typical EM target lesions at each of 4 sites 9 days after ID injection with 10^7 B burgdorferi, *B31 strain passage 4. Left—note the absence of lesions following ID injection of 10^7 avirulent* B burgdorferi, *B31 strain (ATCC).*
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Table 3
Dissemination of B burgdorferi *B31 Passage 4 After ID Inoculation*

(A) **Intradermal inoculation of a total of 4 x 10^7 *B burgdorferi* B31**

Tissues	3 & 5 h	24 h	1 wk	2 wk	3 wk	4 wk
Skin	1/2	2/2	2/2	2/2	2/2	2/2
Ear	0/2	0/2	1/2	1/2	1/2	1/2
Blood	0/2	0/2	0/2	0/2	0/2	0/2
Spleen	0/2	2/2	0/2	0/2	0/2	0/2
Liver	0/2	2/2	0/2	0/2	0/2	0/2
Heart	0/2	1/2	0/2	0/2	0/2	0/2
Popliteal nodes	0/4	0/4	1/2	1/2	2/2	1/2
Stifle joint tissue	ND	ND	ND	1/1	1/2	1/2
Synovial fluid	ND	ND	ND	1/2	0/2	0/2
Conjunctiva	ND	ND	ND	2/2	0/2	0/2
Spinal cord	0/4	0/4	2/2	2/2	2/2	2/2

(B) Intradermal inoculation of a total of 6 x 10^3 *B burgdorferi* B31

Tissues	1 wk	2 wk	3 wk	4 wk	5 wk	8 wk
Skin	9/10	3/3	6/7	3/3	3/4	1/4
Popliteal nodes	ND	2/3	ND	1/3	ND	0/4
Stifle joint tissue	ND	3/3	ND	2/3	ND	0/4
Spinal cord	ND	1/3	ND	0/3	ND	0/4

Data is expressed as No. of rabbits/total No. of rabbits. ND, not done.
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Table 4
Dissemination of Six Strains of B burgdorferi *4 Weeks After ID Inoculation*

	Strain					
	N40	SH-2-82	HB 19	297	CA-2-87	ECM-NY-86
Tissues						
Skin	2/2	2/2	2/2	2/2	2/2	2/2
Popliteal nodes	2/2	1/2	0/2	1/2	0/2	0/2
Stifle joint tissue	1/2	0/2	2/2	0/2	0/2	0/2
Synovial fluid	0/2	0/2	0/2	0/2	0/2	0/2
Spinal cord	2/2	1/2	0/2	1/2	0/2	0/2

For all strains two rabbits were inoculated at each of eight sites with 10^7 organisms per site. Strains used were obtained from skin biopsy cultures of previously infected rabbits and used at in vitro passage 4. Data are expressed as positive rabbits/total rabbits.
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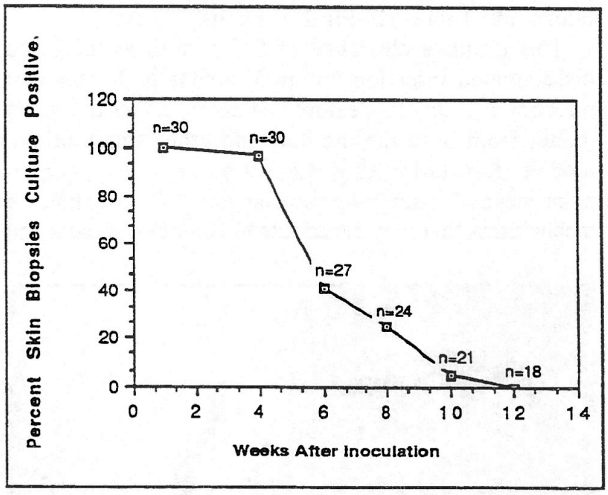

Fig 2. Clearance of B Burgdorferi *B31 from the skin after intradermal injection. Skin biopsies were obtained at each timepoint and cultured in BSK II medium. Graph marker, percentage of culture positive rabbits.*
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infiltration was observed and skin biopsies at the site of inoculation were culture negative. In contrast, a lymphocytic, plasma cell, and macrophage infiltration corresponding to the appearance of culture-positive EM lesions occurred in the naive animals and persisted beyond the time of EM healing and skin resolution. These data suggest that the polymorphonuclear leucocytic host response to *B burgdorferi* is not capable of destroying the organism, that the chronic inflammatory

Table 5
Homologous Challenge of Rabbits Infected ~5 Months Previously With B burgdorferi *B31*

Initial injection		Challenge dose*			
		10^7	10^6	10^5	Culture results
B burgdorferi	Inoculum	EM/total sites			at day 5-7‡
Noninfectious	N/A	6/6	6/6	6/6	+
Heat-killed	4×10^7	3/4	4/4	4/4	+
Lab strain	4×10^7	7/8	6/8	4/8	+
Passage 4	4×10^7	0/4	0/4	0/4	–
	4×10^6	0/2	0/2	0/2	–
	4×10^5	0/2	0/2	0/2	–
	4×10^4	0/2	0/2	0/2	–
	4×10^3	0/4	0/4	0/4	–
Passage 8	4×10^7	0/2	0/2	0/2	–
	4×10^6	2/4	0/2	0/2	–
	4×10^5	1/2	0/2	0/2	–
	4×10^4	0/4	0/4	0/4	–
	4×10^3	3/4	2/4	3/4	–
Passage 27	4×10^7	2/4	2/4	2/4	–
	4×10^6	2/2	0/2	0/2	–
	4×10^5	2/4	2/4	1/4	–
	4×10^4	0/2	0/2	0/2	–
	4×10^3	2/4	2/4	2/4	–
Passage 47	4×10^7	2/2	0/2	0/2	+
	4×10^4	0/2	0/2	0/2	+
	4×10^3	2/2	2/2	2/2	+

**Rabbits were challenged with passage 4 B31. EM lesions were developed at 5–7 d after challenge. ‡Negative biopsied skin cultures obtained at 5–7 d were repeated and found negative at 18, 32, 46, 67, and 81 d after challenge. N/A, not applicable.*
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response may contribute to both EM formation as well as subsequent healing of the lesion and clearance of the spirochetes, and that complete infection-derived immunity is predominately or exclusively antibody mediated.

Another striking feature of the rabbit model is the development of immunity to reinfection in animals challenged with homologous strains of *B burgdorferi*. Within 5 months of initial intradermal infection with three strains of 4×10^7 low-passaged *B burgdorferi*, at a time when both the skin and viscera are cleared of spirochetes, subsequent challenge with as many as 8×10^7 passage 4 homologous spirochetes resulted in no evidence of dermal or disseminated infection[34] (Tables 5 and 6). In the case of the B31 strain, the extent of the protective immunity induced by untreated infection is directly related to the extent of prior in vitro passage[34] (Table 5). Only passage 4 could induce complete protection against EM and dermal infection with as few as 4×10^3 spirochetes.

In contrast, initial infection with passage 27 organisms led to partial protection against EM and complete protection against skin infection. Passage 47 organisms induced no protection against skin infection and partial protection against EM. These data imply that certain determinants required for the production of acquired resistance are lost during in vitro passage. Further, these differences between low and high passage strain B31 may allow the identification of proteins responsible for virulence and/or the induction of complete immunity by the low-passaged spirochetes. Indeed, Skare and his coworkers already have reported significant changes in outer membrane vesicle TX-114 hydrophobic phase proteins during sequential in vitro passage.[36]

Inasmuch as our recent focus has been on structure-

Table 6
Homologous ID Challenge of Rabbits Infected 5 Months Previously with 4 x 10^7 Low Passaged Strains of B burgdorferi

Strain	EM/ Total sites	Skin biopsy cultures: positive rabbits	Visceral cultures: positive rabbits
positive rabbits			
B31	0/16	0/2	0/2
N40	0/16	0/2	0/2
SH-2-82	0/16	0/2	0/2
HB19	8/16	0/2	0/2
297	8/16	0/2	0/2
CA-2-87	0/16	2/2	0/2
ECM-NY-86	0/16	0/2	0/2
Controls			
Heat killed ATCC	16/16	2/2	1/2
Avirulent B31	16/16	2/2	1/2
Noninfected	99/112*	14/14	8/14‡

Two rabbits were challenged at each of eight sites with 10^7 organisms/site. Strains used for challenge were obtained from skin biopsy cultures of previously infected rabbits and used at in vitro passage 4. Data shown for punch biopsy cultures were obtained at 7–11 d after challenge.
*Similar data were obtained at days 21 and 28. Controls include the low passage B31 challenge of those animals previously inoculated with either 2 x 10^7 heat-killed organisms from each strain or given 4 x 10^7 avirulent ATCC B31, and inoculation of two noninfected animals for each strain. Visceral cultures examined in each rabbit were spinal cord, popliteal lymph nodes, and stifle joint tissue. *13 of 16 sites inoculated with the ECM-NY-86 strain failed to develop lesions. ‡See Table 4.*
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Table 7
Hydrophobic OMV Antigens Recognized by Immunoblotting with Adsorbed Sera (Molecular Mass in kD)

Passage 10 B31	Passage 48 B31	Avirulent ATCC B31	Description
16.5		16.5	Oms
18b	18b	18b	Oms, C
19.5a*	19.5a*	19.5a*	Oms, C
19.5c		19.5c	Oms
25c		25c	Oms
28	28		Oms, vsa‡
29a (Osp D)			L, vsa§
29b	29b		Oms, vsa‡
	30		Oms, vsa‡‡
35b			Oms, vsa§
39		39	EF**
40a			Oms, vsa§
40b			Oms, vsa§
40c†			Oms, vsa§
42†			Oms, vsa§
70†			Oms, vsa§

*L, lipoprotein; Oms, candidate outer membrane-spanning protein; C, protein common to all OMV preparations; *for proteins with identical molecular masses, a designates the most acidic protein and the subsequent letters indicate proteins that are more basic; ‡vsa, virulent strain associated protein present in passage 10 and 48 OMV preparations (Figs 3A and B); §vsa, virulent strain associated protein present only in passage 10 OMV preparation (Fig 3A); ‡‡vsa, virulent strain associated protein present only in passage 48 OMV preparation (Fig 3B); †not observed in passage 10 OMV 2-D gold stain; **endoflagellin (EF).*
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function relationships of outer membrane (OM)-spanning proteins, the isolation and purification of *B burgdorferi* OM vesicles (OMV) with porin activity by Skare and his coworkers[36] provided the opportunity to conduct further studies designed to identify, isolate, and purify potentially significant protein antigens from OMV. Serum from a rabbit completely immune to challenge with low-passage B31 *B burgdorferi* was adsorbed with an avirulent ATCC B31 strain isolate to remove antibodies that recognize antigens common to both the virulent and avirulent spirochete.[34] The resulting adsorbed serum contained antibodies specifically enriched for low-passage, virulent *B burgdorferi* antigens and was significantly depleted of antibodies found in the avirulent ATCC B31 strain.

Separation of Triton X-114 detergent phase OM proteins from B31 passage 10 (found to be equivalent to passage 4 in terms of EM induction), B31 passage 48, and the avirulent B31 strain by 2-D gel electrophoresis and subsequent probing of immunoblots with the adsorbed serum, revealed seven antigens found only in passage 10, one of which was identified as OspD36 (Table 7, Fig 3). Two antigens were common to both the passage 10 and passage 48 B31; one was found in passage 48.

The authors have chosen the acronym, Oms, to designate the OM-spanning proteins shared by virulent and avirulent B31 *B burgdorferi*, and Oms^{vsa} for Oms that are virulent-strain associated only. Immunoelectron microscopy using the adsorbed serum paralleled the immunoblot analyses of each of these three isolates[36] (Table 8). The adsorbed serum was found to be more reactive with the virulent isolates than the avirulent isolate.

The observation that the adsorbed serum contains

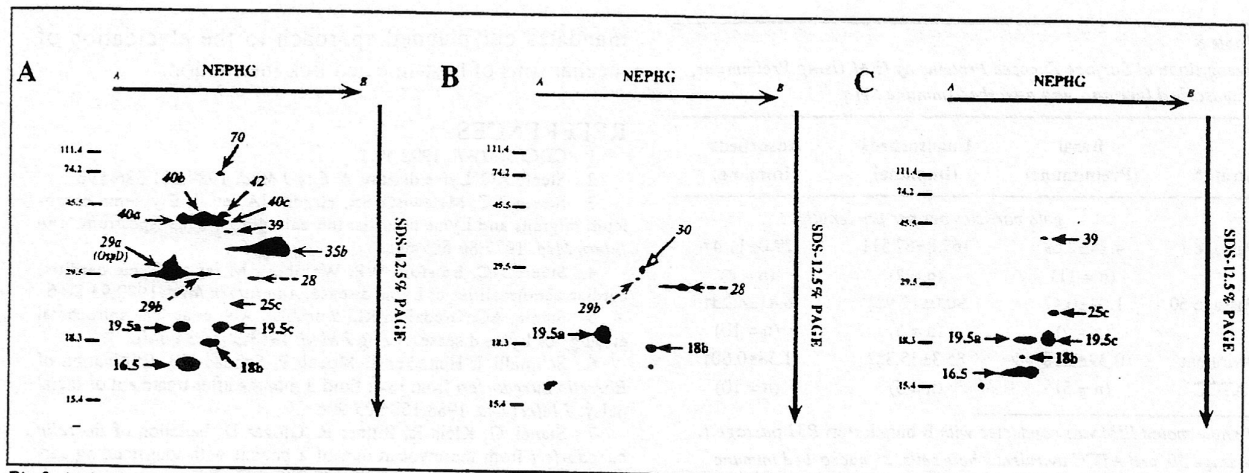

Fig 3. Antigenicity of hydrophobic OM proteins from B burgdorferi *OMV. Identical amounts of Triton X-114 detergent phase OM proteins were separated by 2-D gel electrophoresis and immunoblotted. A and B indicate the acidic and basic ends of the nonequilibrium pH gel (NEPHG), respectively. The immunoblot was incubated in infection-derived rabbit serum adsorbed with avirulent ATCC B31* B burgdorferi. *Lower case letters after the numbers listed distinguish proteins of identical molecular mass with different pI values. The acidic-most spot is designated a; subsequent letter assignments refer to spots with more basic pI values. Numbers to the left indicate the molecular masses of protein markers (kD). (A) OMV derived from 5x10⁹ B31 passage 10* B burgdorferi. *(B) OMV derived from 5x10⁹ B31 passage 48 B. burgdorferi. (C) OMV derived from 5x10⁹ B31 avirulent ATCC* B burgdorferi. *Reprinted with permission of Rockefeller Press.*

antibodies that specifically and preferentially bind the surface of the virulent B31 passage 1 *B burgdorferi* relative to the B31 passage 50 and avirulent ATCC B31 spirochetes provides compelling evidence for presence of antibodies in the adsorbed serum directed specifically against virulent strain-associated antigens with putative virulence and protective immunogenic properties. Of equal significance is the fact that, consistent with the decrease in proteins observed with the antigenic profiles of the OMV preparations from the virulent and avirulent B31 passages[36] (Table 7, Fig 3), the number of gold particles observed for *B burgdorferi* whole cells decreased with increasing in vitro passage[36] (Table 8).

Taken together, the data accrued from the studies using adsorbed serum indicate that Oms^{vsa} are candidate proteins relevant to the pathogenesis and immunology of experimental Lyme disease. Although the Oms^{vsa} described in the studies of Skare and his coworkers are proteins found in *B burgdorferi* after in vitro cultivation, it is conceivable that protein molecules are expressed only in vivo in environments unique to the vertebrate and invertebrate hosts of *B burgdorferi*.[36] Consistent with this possibility is the evidence that a supercoiled plasmid-encoded protein designated EppA and the lipoprotein OspF are expressed only in vivo.[37,38]

ONGOING AND FUTURE STUDIES

The ability to induce EM, skin and disseminated infection, and infection-derived immunity in the rabbit has provided the opportunity to add new dimensions to our approach to the study of Lyme-disease pathogenesis and immunology. The mechanisms that mediate the initial events leading to the production of EM, dissemination, latency, and immunity remain poorly understood. Apparently, the influx of polymorphonuclear leucocytes immediately following infection cannot eradicate the organisms and thus prevent their establishment in the host. The unrestricted multiplication of spirochetes leads to the induction of EM lesions and skin and visceral infections as a result of the contribution of *B burgdorferi* virulence factors and/or the infiltrating chronic inflammatory response to the organism.

Studies along these lines are ongoing or have been designed to clone, sequence, and express the genes that code for the Oms^{vsa} proteins; to examine their putative role as virulence determinants; and to correlate the appearance, persistence, and disappearance of EM and infection with the presence vs absence of T and B lymphocytes, plasma cells, and macrophages.

If T lymphocytes are detected, the presence of CD8$^+$ and CD4$^+$, characterized as TH1 and TH2, will be determined. Experiments to determine the relative distribution of peripheral B and T lymphocytes as well as levels of humoral antibody in relationship to the presence, location, and clearance of the spirochetes also will be conducted.

Although clearance of the initial infection may be mediated by both humoral and cellular mechanisms, the absence of any evidence to indicate that an inflammatory response occurs following the challenge of completely

Table 8
Recognition of Surface Exposed Proteins by IEM Using Preimmune, Unadsorbed Immune, and Adsorbed Immune Sera

Strain*	Basal (Preimmune)	Unadsorbed‡ (Immune)	Adsorbed‡ (Immune)
	gold particles per per μm length§		
Passage 1	4.24±2.69 (n = 11)	167.8±67.511 (n = 7)	29.0±11.4† (n = 8)
Passage 50	1.04±0.47 (n = 5)	90.0±17.9‡‡ (n = 5)	6.41±4.23† (n = 10)
Avirulent ATCC	0.39±0.18 (n = 5)	85.3±35.3‡‡ (n = 5)	1.58±0.60† (n = 10)

**Whole mount IEM was conducted with* B burgdorferi *B31 passage 1, passage 50, and ATCC avirulent whole cells. ‡Unadsorbed immune serum was obtained from a rabbit infected with passage 4 B31* B burgdorferi.[34] *Adsorbed serum was obtained by incubating the unadsorbed serum described above with ATCC avirulent B31* B burgdorferi. *§Values represent the average number of gold particles observed per mm length of the* B burgdorferi *B31 analyzed ±SD; n refers to the number of fields of each sample analyzed under the electron microscope. ‡‡Wilcoxon ranked sum test indicated a significant difference (P = 0.039) when the unadsorbed immune serum was reacted with three strains tested. †Wilcoxon ranked sum test indicated a significant difference (P = 0.0001) when the adsorbed immune serum was reacted with three strains tested.*
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immune animals strongly implies that exogenous resistance is mediated exclusively by humoral antibody. Passive protection, opsonophagocytic, complement-dependent borreliacidal, and adherence and invasin inhibition assays have been planned in an effort to confirm this hypothesis.

A highly significant advantage of the rabbit vs the rodent models is the fact that the former permits the definition of vaccine efficacy against EM as well as skin and disseminated infection. If a test vaccinogen exhibits a high degree of—but not complete—protection against skin and disseminated infection, an evaluation must be made of its ability to protect against EM in the susceptible animals. Protection against only EM will result in an undetected, "masked" latent state with the potential for reactivation of the disease. Studies to determine the protective immunogenicity of purified Oms^{vsa} proteins as well as a recombinant OspA vaccine being evaluated in humans are underway.

The rabbit model using needle inoculation has provided and continues to provide data that contribute significantly to our understanding of the pathogenesis and immunology of Lyme disease. However, the necessity to compare and contrast the events following intrdermal infection with the natural method of tick transmission, mandates our planned approach to the elucidation of mechanisms of host-infected tick interaction.

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