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ORIGINAL INVESTIGATION

Annette Pohl-Koppe · Angelika Kaunicnik · Bettina Wilske

Characterization of the cellular and humoral immune response to outer surface protein C and outer surface protein 17 in children with early disseminated Lyme borreliosis

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Abstract OspC and Osp17 are immunodominant proteins of *Borrelia burgdorferi* eliciting a clear humoral immune response in adult patients with systemic Lyme disease. In this study, the cellular immune response to *B. burgdorferi* and the major outer surface proteins OspC and Osp17 was investigated in children during the course of early disseminated *B. burgdorferi* infection. Lymphoproliferative responses to recombinant proteins were compared to the protein-specific humoral immune reaction. OspC induced a clear antibody response but elicited an even stronger cellular immune response. In contrast, a cellular as well as humoral immune reaction to Osp17 was only rarely detected. Follow-up examinations demonstrated that the lymphoproliferative response to *B. burgdorferi* and OspC persisted for several months after antibiotic therapy. Here, we show that in early disseminated Lyme disease of childhood, OspC is a potent antigen influencing both the humoral and cellular immunity, while Osp17 plays only a minor role in immune activation.

Keywords *Borrelia burgdorferi* · OspC · Osp17 · Humoral immunity · Cellular immunity

Introduction

Lyme borreliosis is a multisystemic disease caused by *Borrelia burgdorferi*. In Europe, three species are known to be pathogenic for humans: *B. burgdorferi* sensu stricto, *B. afzelii* and *B. garinii* [1, 5, 34]. Primary contact with *B. burgdorferi* may lead either to erythema migrans (EM), which represents a localized infection (stage I), or may progress to early disseminated infection (stage II) with involvement of the skin (multiple EM, Borrelia lymphocytoma) [24], central nervous system (CNS) (facial palsy, aseptic meningitis, meningopolyradiculitis), joints (acute Lyme arthritis) and heart (myocarditis). Late stage disease (stage III) that is rarely seen in pediatric patients is characterized by chronic *B. burgdorferi* infection of the skin (acrodermatitis chronica atrophicans), joints (chronic Lyme arthritis) or CNS (encephalopathy, encephalomyelitis) [6]. The diagnosis of Lyme borreliosis is confirmed by serology in stage II and III disease. Borrelia-specific antibodies are found in only 20–50% of patients with EM (stage I), whereas in stage II and III disease, antibodies are demonstrated in 70–90% and 90–100% of cases, respectively [32]. Although in animal models a protective effect of Borrelia-specific antibodies could clearly be demonstrated [2, 29], systemic stages of disease may develop in the presence of a vigorous humoral immune response. Thus, the cellular immune response may play a particularly important role in the pathogenesis of Lyme disease. An increased cellular reactivity measured by lymphoproliferation or reactivity of T cell lines and clones to Borrelia spirochetes has been demonstrated in adult patients with various cutaneous manifestations, neuroborreliosis and Lyme arthritis [3, 4, 8, 10, 25, 26, 41] as well as in children with Lyme arthritis [17]. As lymphoproliferative studies revealed a large variability of T cell responses in seropositive and seronegative patients, lymphoproliferation had been assessed to individual borrelial proteins such as outer surface protein A (OspA), OspB, p41, p55/58 and p66 in adult patients with Lyme arthritis [19, 21, 22, 41]. So far, attempts to associate the cellular immune response to certain Borrelia proteins with particular manifestations of Lyme disease have not been convincing. To which extent borrelial proteins elicit a cellular immune response in

B. Wilske (✉)
Max-von-Pettenkofer-Institut für Hygiene
und Medizinische Mikrobiologie,
Ludwig-Maximilians-Universität,
Pettenkoferstrasse 9a, 80337 München, Germany
E-mail: Bettina.Wilske@mvp-bak.med.uni-muenchen.de
Tel.: +49-89-51605231
Fax: +49-89-51604757

A. Pohl-Koppe · A. Kaunicnik
Department of Infectious Diseases,
Children's Hospital, Ludwig-Maximilians-Universität,
Lindwurmstrasse 4, 80337 München, Germany

comparison to the humoral immune response has not yet been investigated. Furthermore, only few data exist on the immune reaction to *B. burgdorferi* in primary Borrelia infection in childhood [17].

In this study, we were interested in examining the cellular immune response to *B. burgdorferi* spirochetes and the two outer surface proteins OspC [35] and Osp17 [18] in children with early disseminated Lyme borreliosis. Cellular immune responses measured by lymphoproliferation were compared to humoral immune responses assayed by immunoblot testing. Both Osps are immunodominant proteins eliciting a strong humoral immune response in adult patients with systemic Lyme disease [15, 37, 40]. OspC expressed during primary infection is the immunodominant protein of the early humoral immune response (preferentially IgM antibodies), whereas Osp17 is expressed predominantly during late stages of disease eliciting preferentially IgG antibodies in only some of the patients with stage II, but regularly in patients with stage III manifestations [15, 40]. While the serological response to OspC and Osp17 has been well defined in adult patients, only little is known in pediatric patients. Furthermore, in children it is unclear to which extent OspC and Osp17 induce a cellular immune response during the natural course of primary *B. burgdorferi* infection and whether protein-specific T cell reactivity is maintained during the course of disease.

Material and methods

Patients

Study group

Between May 1997 and December 1999, 28 children with Lyme borreliosis stage II were enrolled at the Children's Hospital of the Ludwig-Maximilians-University in Munich. Of these children, 15 were diagnosed with acute neuroborreliosis based on cerebrospinal fluid (CSF) pleocytosis and Borrelia-specific antibodies (see serology). Six children were diagnosed with Lyme arthritis of the knee ($n=4$), ankle ($n=1$) and elbow ($n=1$). Seven children developed cutaneous manifestations such as Borrelia lymphocytoma on the ear ($n=2$), breast areola ($n=2$), cheek ($n=1$) and neck ($n=1$) or multiple EM ($n=1$) (Table 1). The mean age was 8.5 years. A tick bite was recalled in 11 children. The diagnosis and treatment of Lyme borreliosis was established according to the guidelines of the German pediatric society of infectious diseases [6]. After informed consent was obtained, blood was drawn for the assessment of humoral and cellular immune responses and before antibiotic therapy was initiated. In 10 children, the humoral and cellular immune responses to *B. burgdorferi* was reexamined between 2 and 6 months (mean 3 months) after successful antibiotic treatment.

Control group

As controls, 16 children (8 girls and 8 boys) aged 1–12 years (mean 8.2 years) were enrolled. Ten children were healthy, 1 child had an unspecific febrile illness, 2 children had hepatitis C, 1 child had arthritis of the right knee and 2 children had idiopathic facial palsy. Borrelia serology was negative in all children. Additionally, a Borrelia-specific PCR in the joint fluid was negative in the child with gonarthritis and there were no Borrelia-specific antibodies in the CSF of the 2 children with facial palsy.

Serology

Serological analysis was performed by screening antibodies against *B. burgdorferi* in a commercially available enzyme-linked immunosorbent assay (ELISA) (Dade-Behring, Marburg, Germany) and a standard indirect immunofluorescence assay (IFA) as described previously [36]. In both assays, *B. burgdorferi* strain PKo served as antigen. Preabsorption with *Treponema phagedenis* was used to avoid false-positive results. Test results were considered positive when an absorbance of ≥0.5 for ELISA IgM, ≥10 U/ml for ELISA IgG and ≥1:32 for IFA IgM and IgG were measured. Borderline or positive serological results were confirmed by immunoblot assays with the recombinant Borrelia proteins OspC, Osp17, p39, internal fragment of p41, p58 and p100 [40]. Expression and purification of recombinant proteins were performed as previously described [18, 40]. In all children, diagnosis of Lyme disease was confirmed by either positive IgG and/or IgM antibodies against *B. burgdorferi*. In patients with neuroborreliosis, intrathecal antibody production against *B. burgdorferi* (CSF/serum index) was determined as previously described [33]. A CSF/serum index of ≥2.0 was considered positive. In 11 of 15 children with neuroborreliosis, *B. burgdorferi*-specific antibodies were detected in the CSF. Due to limited amounts of CSF, intrathecal synthesis of these antibodies could only be confirmed by a positive CSF/serum index in 4 of 11 children. In 1 of 3 children without CSF antibodies, a positive *B. burgdorferi* polymerase chain reaction (PCR) in the CSF confirmed the diagnosis of neuroborreliosis in the absence of intrathecal antibody synthesis. In 2 other children, who were negative for *B. burgdorferi*-specific antibodies in the CSF, and in 1 child, in whom no CSF could be examined, the diagnosis of neuroborreliosis was established by typical clinical manifestations and positive serum IgM titers. Additionally, 1 of these children had a clinical manifestation of EM. In children with Lyme arthritis, the diagnosis was based on the evidence of positive serum antibodies. A joint puncture was not clinically indicated in any of the children.

Lymphoproliferative assays

Lymphoproliferative assays were performed on heparinized blood as previously described [13]. Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Hypaque density gradient centrifugation (Pharmacia, Piscataway, NJ) and plated out at 5×10^4 cells/well in RPMI 1640, containing 4 mM L-glutamine, 25 mM HEPES buffer, 50 U/ml penicillin, 50 μg/ml streptomycin (Whittaker, Walkersville, MD) and 10% autologous heat-inactivated serum. After primary experiments to establish optimal antigen concentrations had been performed, T cell cultures were stimulated in duplicates with either lysate of whole, sonicated *B. afzelii* spirochetes (strain PKo) at 10 μg protein/ml, lysates of whole *B. garinii* spirochetes (strain PBi) at 10 μg protein/ml, or recombinant OspC and Osp17 of *B. afzelii* (strain PKo) [18, 37], both at 50 μg protein/ml. Both recombinant proteins were the same as used in immunoblots. As controls, PBMC were incubated with medium alone and with tetanus toxoid (TT) at 12.5 Lf/ml (kindly provided by Dr. Hungerer, Dade-Behring, Marburg, Germany). After 5 days, [^{3}H]thymidine was added to the wells and T cell proliferation was measured by incorporation of [^{3}H]thymidine after 18 h. Proliferation is indicated as mean cpm measured in the presence of antigen minus mean cpm with no antigen present (Δ cpm). Lymphoproliferation was only considered significant when cpm was less than 500 in the condition without antigen and Δ cpm was greater than 1,000.

Approval of the study

This study has been approved by the ethical committee of the Ludwigs-Maximilians-University, Munich, Germany.

Table 1 Clinical characteristics of patients with Lyme borreliosis stage II (*IFA* immunofluorescence assay, *EM* erythema migrans, *Cefo* Cefotaxime, *Ceftr* Ceftriaxone, *Cefur* Cefuroxime-Axetil, *Amox* Amoxicilline, *Doxy* Doxycycline, *Erythro* Erythromycin)

Patient	Age (years)	Sex	Diagnosis	*B. burgdorferi* ELISA		*B. burgdorferi* ELISA		CSF/serum	Antibiotic therapy and duration
	Range (3–14)	13 F/15 M		IgM in serum	IgG	IgM in CSF	IgG	IgG index	
1	3	F	Facial palsy[b]	0.342	Negative	Reactive	Negative	ND	Cefo 14 days
2	5	F	Facial palsy + EM	1.399	Negative	Reactive	Negative	nd	Ceftr 12 days
3[a]	7	F	Facial palsy	0.656	10	nd	nd	nd	Ceftr 14 days
4	8	M	Facial palsy + EM	0.679	17	Negative	Negative	nd	Cefo 14 days, Ceftr 6 days
5	9	F	Facial palsy	1.022	15	Reactive	Reactive	5.1	Cefo 14 days
6	10	M	Facial palsy	Negative	Negative	Reactive	Negative	nd	Cefo 14 days
7[a]	11	M	Facial palsy	0.839	Negative	Negative	Negative	nd	Cefo 8 days, Ceftr 18 days
8[a]	6	M	Aseptic meningitis	0.887	67	Reactive[c]	Reactive	5.4	Cefo 10 days, Cefur 4 days
9	8	F	Aseptic meningitis	0.412	Negative	Negative	Negative	nd	Ceftr 21 days
10[a]	8	M	Aseptic meningitis	1.369	Negative	Reactive	Reactive	nd	Cefo 9 days, Ceftr 4 days
11	10	F	Aseptic meningitis	0.377	>250	Reactive[c]	Reactive	nd	Ceftr 14 days
12[a]	13	F	Aseptic meningitis	2.062	6	Reactive	Reactive	nd	Ceftr 14 days
13	9	M	Bannwarth syndrome	Negative	Negative	Negative	Reactive	18.1	Ceftr 14 days
14[a]	9	F	Bannwarth syndrome	0.551	Negative	Reactive	Negative[d]	35.8[d]	Cefo 5 days, Ceftr 7 days
15	10	M	Bannwarth syndrome + EM	1.748	Negative	Reactive	Negative	nd	Ceftr 12 days
16	9	M	Lyme arthritis	0.590	>410				Cefur 5 days, Ceftr 12 days
17	9	M	Lyme arthritis	Negative	210				Amox 21 days
18[a]	10	M	Lyme arthritis	Negative	>480				Ceftr 10 days
19	11	M	Lyme arthritis	0.368	>280				Amox 28 d
20	12	M	Lyme arthritis	Negative	>290				Ceftr 14 days
21	14	F	Lyme arthritis	Negative	>520				Doxy 35 days, Ceftr 33 days
22	5	M	Multiple EM	2.646	12				Amox 21 days
23[a]	5	M	Lymphocytoma	Negative	10				Amox 21 days
24[a]	5	F	Lymphocytoma	Negative	>350				Erythro 7 days, Amox 21 days
25	8	F	Lymphocytoma	Negative	135				Cefur 28 days
26	9	F	Lymphocytoma	Negative	63				Cefur 14 days
27	10	M	Lymphocytoma	0.691	23				Amox 21 days
28[a]	8	F	Lymphocytoma	Negative	250				Amox 10 days, Cefur 53 days, Ceftr 14 days

[a]Reexamination after 2–6 months
[b]*B. burgdorferi*-specific PCR in the positive CSF (*B. garinii*)
[c]Oligoclonal bands in positive CSF
[d]Borrelia-specific CSF IgG antibodies in IFA were 1:8, CSF/serum index calculated with IFA titers

Statistics

Statistical analysis was performed with the Mann-Whitney Test.

Results

Immune response to *B. burgdorferi* lysate antigens and TT

The lymphoproliferation to lysate antigens of *B. afzelii* and *B. garinii* in 28 children with early disseminated Lyme borreliosis were compared to 16 age-matched seronegative controls. In preceding experiments, we demonstrated by flow cytometry that after 6 days of antigenic stimulation, 50–60% of PBMC were $CD4^+$ T lymphocytes, 15–25% were $CD8^+$ T lymphocytes and 10–20% were B lymphocytes (data not shown). The mean lymphoproliferation to *B. afzelii* and *B. garinii* differed significantly between Lyme patients and controls (Table 2). However, there were few individuals with Lyme borreliosis, who showed only a weak cellular immune response to the lysate antigens and vice versa, few controls displayed a significant reaction to the lysate antigens (Fig. 1). As a control antigen, the recall antigen TT was used. All patients and controls had a history of

Table 2 Lymphoproliferation to borrelial antigens and TT (*Ab* antibody, *TT* tetanus toxoid)

Antigen	Immunoblot	Lyme borreliosis patients (samples)	Controls patients (samples)	Mean lymphoproliferation		
				Lyme borreliosis (Δ cpm ± SD)	Controls (Δ cpm ± SD)	*P*
B. garinii		28 (37)	14 (14)	19,950 ± 14,864	5,979 ± 5,526	0.001
B. afzelii		28 (39)	16 (16)	19,946 ± 15,132	10,878 ± 10,126	0.03
OspC	OspC Ab positive or negative	28 (39)	16 (16)	3,822 ± 3,372	1,566 ± 1,591	0.002
OspC	OspC Ab positive	12 (17)	16 (16)	4,944 ± 4,123	1,566 ± 1,591	0.006
OspC	OspC Ab negative	16 (22)	16 (16)	2,866 ± 2,190	1,566 ± 1,591	0.04
Osp17	Osp17 Ab positive or negative	25 (31)	13 (13)	251 ± 463	68 ± 178	0.07
Osp17	Osp17 Ab positive	6 (8)	13 (13)	260 ± 573	68 ± 178	0.07
Osp17	Osp17 Ab negative	19 (23)	13 (13)	247 ± 417	68 ± 178	0.09
TT		27 (36)	16 (16)	17,305 ± 3,782	13,909 ± 1,600	0.29

TT vaccination. As expected, there were no significant differences in the lymphoproliferative response to TT (Table 2, Fig. 1).

Immune response to OspC and Osp17

The cellular immune response to OspC and Osp17 was examined in 28 children with Lyme disease and compared to 16 controls. The mean lymphoproliferation to OspC was significantly increased in children with Lyme disease. This difference was still observed when the group of Lyme patients was divided into OspC antibody-positive and OspC antibody-negative individuals, although there was a stronger lymphoproliferative response to OspC in the OspC antibody-positive individuals (Table 2). In contrast to OspC, significant lymphoproliferation to Osp17 was rarely detected. The mean Osp17-specific proliferation of all examined samples did not differ significantly between Lyme patients and controls (Table 2). In 25 Lyme patients and 13 controls, lymphoproliferative responses could be assessed to both Osp17 and OspC. While OspC appeared as an immunodominant antigen inducing a strong cellular immune response in 21 of 25 patients, only 3 of 21 patients also reacted to Osp17. In 4 of 25 patients, no lymphoproliferation was observed to either OspC or Osp17. No patient showed a lymphoproliferative response to only Osp17 (Fig. 2).

In each individual, the cellular immune response to OspC and Osp17 was compared to the humoral immune response. Of the 28 Lyme disease patients 12 (43%) had OspC-specific antibodies, with IgM antibodies in 8 patients, IgG antibodies in 2 patients, and IgM and IgG antibodies in 2 patients. These children were diagnosed with multiple EM (patient 22), borrelia lymphocytoma (patients 24 and 27), facial palsy (patients 2 and 4), aseptic meningitis (patients 10 and 12), meningopolyradiculitis (patient 15) and acute Lyme arthritis (patients 16, 19, 20 and 21). OspC-specific lymphoproliferation was observed in 11 of 12 OspC antibody-positive patients and in 13 of 16 OspC antibody-negative patients, indicating that OspC is eliciting not only a humoral but an even stronger cellular immune response (Table 3). Reactivity to Osp17 was clearly reduced in comparison to OspC. Of 25 patients, 6 (24%) had Osp17-specific IgG antibodies. These children were diagnosed with borrelia lymphocytoma (patients 27 and 28), multiple EM (patient 22), aseptic meningitis (patient 11) and acute Lyme arthritis (patients 16 and 18). OspC- and Osp17-specific antibodies were only present in three patients (patients 16, 22 and 27). Osp17-specific lymphoproliferation was observed in only 1 of 6 children with Osp17 antibodies (patient 28) and in 2 of 19 children without Osp17 antibodies (patients 9 and 12) (Table 3).

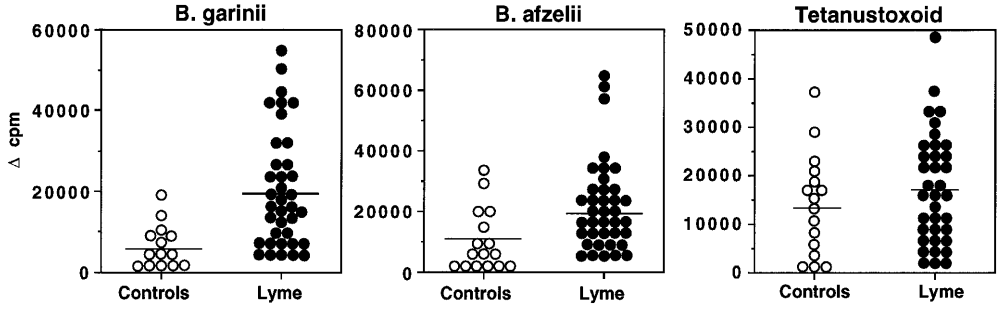

Fig. 1 Lymphoproliferation to *Borrelia garinii*, *B. afzelii* and TT. PBMC of children with acute systemic Lyme borreliosis (*black circles*) and controls (*white circles*) were stimulated with lysate antigens of *B. burgdorferi* or TT for 6 days. Proliferation was measured by [^{3}H]thymidine incorporation. The mean proliferation to *B. burgdorferi*, but not to TT, differed significantly between patients and controls (see Table 2) (*TT* tetanus toxoid, *PBMC* peripheral blood mononuclear cells)

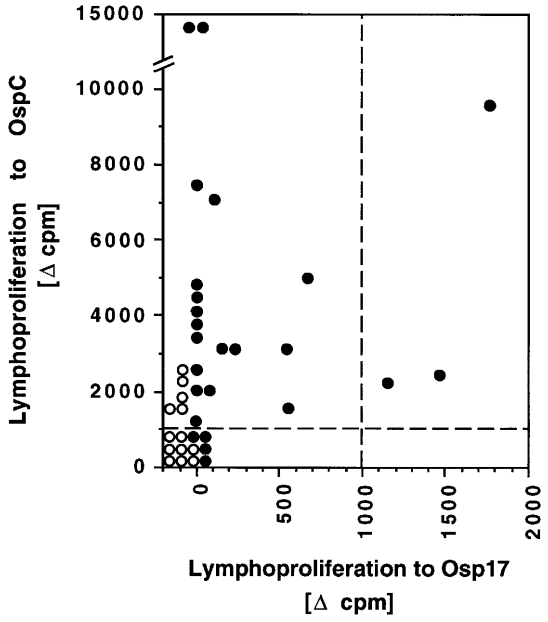

Fig. 2 Lymphoproliferation to OspC and Osp17. PBMC of children with acute systemic Lyme borreliosis (*black circles*) and controls (*white circles*) were stimulated with recombinant OspC and Osp17 for 6 days. Proliferation was measured by [^{3}H]thymidine incorporation. A cellular immune response to OspC was observed in 21 of 25 patients, but only 3 of 21 patients reacted also to Osp17. A weak lymphoproliferative response to OspC but not to Osp17 was observed in 5 of 13 controls

Table 3 Humoral and cellular immune response to OspC and Osp17. Twenty-eight children with Lyme borreliosis were examined for OspC-specific and 25 for Osp17-specific immune responses. Antibodies to OspC and Osp17 were tested by immunoblot analysis. Lymphoproliferation was assessed after 6 days of antigenic stimulation by [^{3}H]thymidine incorporation

Antigen	Lymphoproliferation in antibody-positive patients		Lymphoproliferation in antibody-negative patients	
	Positive (%)	Negative (%)	Positive (%)	Negative (%)
OspC	11/12 (91.2)	1/12 (8.3)	13/16 (81.2)	3/16 (18.8)
Osp17	1/6 (16.7)	5/6 (83.3)	2/19 (10.5)	17/19 (89.5)

Cellular and humoral immune responses to *B. burgdorferi* after antibiotic therapy

In 10 children, the cellular and humoral immune responses to *B. burgdorferi* were tested after 2–6 months. Clinically, all children had recovered completely after antibiotic treatment. There were no significant changes of preexisting IgG titers in 4 children (patients 8, 18, 24 and 28). Three children showed a persistence of high level IgM titers in the absence of IgG seroconversion (patients 3, 12 14). Another 3 children were completely seronegative at follow-up with a loss of IgM titers and no IgG seroconversion (patients 7 and 10) and a loss of initially low IgG titers (patient 23). As a loss of initially positive *B. burgdorferi*-specific antibody titers, particularly in patients with successful antibiotic treatment, had been described earlier [7, 32], we wanted to determine whether the cellular immune responses to *B. burgdorferi* had been maintained during the course of disease. Thus, the lymphoproliferation to *B. burgdorferi* antigens and TT was assessed. As it is shown in Fig. 3, lymphoproliferative responses to *B. afzelii* persisted in all but one patient (Fig. 3A). Lymphoproliferative responses to OspC were initially observed in 6 of 10 patients, although an antibody response to OspC was present in only 3 children; at follow-up a persistence of reactivity was seen in all these patients. Additionally, 2 other children without OspC-specific lymphoproliferation at the time of diagnosis had developed lymphoproliferative responses to OspC in the absence of OspC-specific antibodies at follow-up (Fig. 3B). Lymphoproliferative responses to TT were maintained in all children (Fig. 3C).

Discussion

After primary *B. burgdorferi* infection systemic Lyme disease may develop despite a clear humoral immune response suggesting that other mechanisms of the immune defense such as the cellular immune response play an important role in the manifestation of disease. In this study, we therefore characterized the cellular immune response to *B. burgdorferi* and its outer surface proteins OspC and Osp17 in children with early disseminated Lyme borreliosis.

Lymphoproliferation to lysate antigens

Lysate preparations of *B. afzelii* and *B. garinii* were chosen as these two species of *B. burgdorferi* sensu lato have been isolated most frequently from European patients [1, 5, 32, 38]. The mean lymphoproliferation to these lysate antigens was increased in children with early disseminated Lyme borreliosis in comparison to seronegative controls. Similar results have been observed by others. Significant differences in the *B. burgdorferi*-specific lymphoproliferation were demonstrated between adult patients with Lyme disease and seronegative controls [10, 21]. Additionally, in pediatric patients with Lyme arthritis, an increased T cell reactivity to *B. burgdorferi* whole cell lysate antigen was shown [17]. However, a weak to moderate lymphoproliferative response to *B. burgdorferi* in some seronegative controls has been observed in this study and by others [28, 43]. This might be explained by usage of whole or sonicated *B. burgdorferi* spirochetes, which contain certain antigens such as heat-shock proteins that share cross-reactive epitopes with antigens of other bacteria [14].

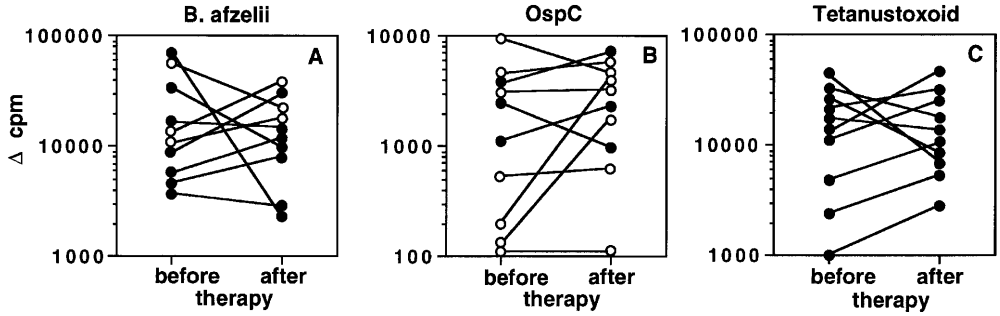

Fig. 3 Lymphoproliferation to *B. afzelii*, OspC and TT before and after antibiotic therapy. PBMC of ten children with Lyme disease stage II were stimulated with *B. afzelii*, OspC and TT before initiation of antibiotic treatment and between 2 and 6 months after completed therapy. **A** A persistence or mild decrease of *B. burgdorferi*-specific antibody titers was observed in seven children (*black circles*), while a complete loss of *B. burgdorferi*-specific antibodies was demonstrated in three children (*white circles*). **B** Three patients had OspC-specific antibodies (*black circles*) and seven patients had no OspC-specific antibodies in immunoblot testing (*white circles*). Six children showed a significant lymphoproliferation to OspC at the time of diagnosis, additional two children developed lymphoproliferative responses to OspC after treatment. **C** The lymphoproliferation to TT appeared to be unaffected by antibiotic therapy

Cellular and humoral immune response to recombinant outer surface proteins

As lymphoproliferation to borrelial lysate antigens was also observed in seronegative individuals, it was of interest to examine the lymphoproliferative response to single borrelia proteins. While in the past extensive studies have focused on T cell recognition of OspA predominantly in patients with Lyme arthritis [19, 21, 22, 41], lymphoproliferation to OspC and Osp17 have not been examined so far. This may be explained in part by the fact that initially OspA was considered a primary vaccine candidate [31] but also by the fact that recombinant OspC and especially Osp17 became available at considerably later time points. Meanwhile, it has been shown that OspC is also an effective vaccine in the animal model [27] and that immune serum against OspC has a therapeutic effect on the resolution of Lyme arthritis in SCID mice [42]. Furthermore, a recombinant vaccine based on OspC is currently being developed industrially. As OspC is up-regulated and OspA is down-regulated during the bloodmeal of the tick [11, 30], OspC-expressing borrelia spirochetes infect the host, thus leading to a primary humoral immune response directed predominantly to OspC [12, 15, 32, 37]. In our study, 10 of 28 children (35.7%) had OspC-specific IgM antibodies and 4 of 28 children (14.3%) had OspC-specific IgG antibodies. These frequencies are almost equally observed in adult patients with acute neuroborreliosis, where 27% have IgM antibodies and 17% have IgG antibodies to OspC [15]. Moreover, in our study OspC not only induced a clear humoral immune response but also elicited an even stronger cellular immune response. In Lyme patients, 91% of those with OspC antibodies and 75% of those without OspC antibodies showed a significant lymphoproliferation to OspC. This characterizes OspC as a potent stimulus eliciting a cellular immune response.

In contrast to OspC, a humoral immune response to Osp17 was less frequently detected during early disseminated infection [15, 40]. Moreover, Osp17 did not induce a relevant lymphoproliferative response in our pediatric patients. Only 27% of adult patients with stage II disease, but 95% of patients with stage III disease, were seropositive for Osp17 in the conventional immunoblot [15]; similar data were subsequently obtained for the recombinant Osp17-immunoblot [40]. In analogy to the findings in adult patients, 24% of our children had Osp17-specific antibodies. Since Osp17-specific antibodies are predominantly detected during late stages of the disease, it appears that Osp17 is up-regulated during infection in the mammal host. Overall, in pediatric patients with early disseminated Lyme borreliosis, Osp17 appeared as a weak stimulus for T cell proliferation with lymphoproliferation in only 12% of examined children.

The recombinant proteins OspC and Osp17 were cloned from *B. afzelii*, strain PKo, as this is one of the best characterized strains of our laboratory. An assessment of the immune response to recombinant OspC and Osp17 derived from other European strains was not possible due to the limited amount of blood that was drawn from children. As a significant heterogeneity has been described for OspC in European strains [35, 38, 39], one might reason that in this study an immune response to OspC and/or Osp17 was detected only in patients with *B. afzelii* infection. However, concerning OspC, we found that the IgM response is primarily directed against conserved epitopes [37], a finding confirmed also by Mathiesen et al. [23]. For Osp17, in can not be ruled out that heterogeneity may play a greater role compared to OspC. In our study, all patients, who showed a lymphoproliferative response to whole cell lysate of *B. afzelii* also recognized *B. garinii*.

Persistence of the immune response after antibiotic treatment

Lymphoproliferation and antibody response were examined during the course of infection in ten children. A long persistence of IgM antibodies accompanied by a late seroconversion to IgG, as observed in three of ten

patients, has also been described by others [7]. Early antibiotic therapy may critically influence the development of humoral immune responses, as demonstrated in three of our children who showed lack of IgG seroconversion with a loss of IgM antibodies. This has been observed previously in patients with antibiotic treatment that had been given during the early course of infection [7, 9]. It is likely that these patients are susceptible for reinfection. While the humoral immune response is obviously influenced by early antibiotic therapy, few data exist indicating that the cellular immune response seems to be unaffected [10, 17]. This previously observed phenomenon was confirmed in our study, as lymphoproliferative responses to *B. afzelii* and OspC at primary examination had still been maintained several months later in all but one children. Furthermore, two of four children without OspC antibodies, who initially did not show lymphoproliferation to OspC, reacted to OspC at follow up. That an initially negative T cell response to *B. burgdorferi* could still become positive in children even after antibiotic therapy has also been observed by others [17]. Our results, however, are in contrast to observations in adult patients with Lyme arthritis [20] and EM [16], who showed a decline of the cellular immune response after antibiotic treatment. This may be explained by different time intervals between initial and follow-up examinations. While the control examination was performed after an average of 3 months in our study and that by Huppertz et al. [17], in other studies the control examinations were conducted after more than 6 months [20, 16].

In summary, in this study we were able to demonstrate that in children with early disseminated Lyme borreliosis there is a significant lymphoproliferative response not only to sonicated *B. burgdorferi* spirochetes but also to OspC. OspC, but not Osp17, induced a clear humoral as well as cellular immune response. Follow-up examinations several months after antibiotic therapy revealed persistence of the lymphoproliferative response to *B. burgdorferi* and OspC. The results of this study correspond to the observation that OspC is predominantly expressed during acute stages of Lyme borreliosis, while Osp17 is mainly expressed during late-stage disease.

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References

1. Baranton G, Postic D, Saint Girons I, Boerlin I, Piffaretti J-C, Assous M, Grimont PA (1992) Delineation of *Borrelia burgdorferi* sensu stricto, *Borrelia garinii* sp. nov., and group VS461 associated with Lyme borreliosis. Int J Syst Bacteriol 42:378–383
2. Barthold SW, Souza MS de, Feng S (1996) Serum-mediated resolution of Lyme arthritis in mice. Lab Invest 74:57–67
3. Buchner SA, Rufli T (1987) Erythema chronicum migrans: evidence for cellular immune reaction in the skin lesion. Dermatologica 174:144–149
4. Buchner SA, Lautenschlager S, Itin O, Bircher A, Erb P (1995) Lymphoproliferative responses to *Borrelia burgdorferi* in patients with erythema migrans, acrodermatits chronica atrophicans, lymphadenosis benigna cutis and morphea. Arch Dermatol 131:673–677
5. Canica MM, Nato F, Du Merle L, Mazie JC, Baranton G, Postic D (1993) Monoclonal antibodies for identification of *Borrelia afzelii* sp. nov. associated with late cutaneous manifestations of Lyme borreliosis. Scand J Infect Dis 25:441–448
6. Christen HJ (2000) Lyme-Borreliose. In: Deutsche Gesellschaft für pädiatrische Infektiologie (Hrsg) DGPI Handbuch 1997: Infektionen bei Kindern und Jugendlichen. Futuramed, München, pp 418–424
7. Christen HJ, Hanefeld F, Eiffert H, Thomssen R (1993) Epidemiology and clinical manifestations of Lyme borreliosis in childhood. A prospective multicenter study with special regard to neuroborreliosis. Acta Paediatr Suppl 386:S1–76
8. Dattwyler RJ, Volkman DJ, Halperin JJ, Luft BJ, Thomas J, Golightly MG (1988) Specific immune responses in Lyme borreliosis: Characterization of T cell and B cell responses to *Borrelia burgdorferi*. Ann N Y Acad Sci 539:93–102
9. Dattwyler RJ, Volkman DJ, Luft BJ, Halperin JJ, Thomas J, Golightly MG (1988) Seronegative Lyme disease. N Engl J Med 319:1441–1446
10. Dressler F, Yoshinari NH, Steere AC (1991) The T cell proliferative assay in the diagnosis of Lyme disease. Ann Intern Med 115:533–539
11. Fingerle V, Liegl G, Munderloh U, Wilske B (1998) Expression of outer surface proteins A and C of *Borrelia burgdorferi* in *Ixodes ricinus* ticks removed from humans. Med Microbiol Immunol (Berl) 187:121–126
12. Fung BP, McHugh GL, Leong JM, Steere AC (1994) Humoral immune responses to outer surface protein C of *Borrelia burgdorferi* in Lyme disease: role of the immunoglobulin M response in the serodiagnosis of early infection. Infect Immun 62:3213–3221
13. Hafler JP, Pohl-Koppe A (1998) The cellular immune response to *Bordetella pertussis* in two children with whooping cough. Eur J Med Res 3:523–526
14. Hansen K, Bangsborg JM, Fjordvang H, Pedersen NS, Hindersson P (1988) Immunochemical characterization and isolation of the gene for a *Borrelia burgdorferi* immunodominant 60 kDa antigen common to a wide range of bacteria. Infect Immun 56:2047–2053
15. Hauser U, Lehnert G, Lobentanzer R, Wilske B (1997) Interpretation criteria for standardized Western blots for three European species of *Borrelia burgdorferi* sensu lato. J Clin Microbiol 35:1433–1444
16. Horowitz HW, Pavia CS, Bittker S, Forseter G, Cooper D, Nadelman RB, Byrne D, Johnson RC, Wormser GP (1994) Sustained cellular immune responses to *Borrelia burgdorferi*: lack of correlation with clinical presentation and serology. Clin Diagn Lab Immunol 1:373–378
17. Huppertz H-I, Mösbauer S, Busch D, Karch H (1996) Lymphoproliferative responses to *Borrelia burgdorferi* in the diagnosis of Lyme arthritis in children and adolescents. Eur J Pediatr 155:297–302
18. Jauris-Heipke S, Rossle B, Wanner G, Habermann C, Rossler D, Fingerle V, Lobentanzer R, Pradel I, Hillenbrandt B, Schult-Spechtel U, Wilske B (1999) Osp17, a novel immunodominant outer surface protein of *Borrelia afzelii*: recombinant expression in *Escherichia coli* and its use as a diagnostic antigen for serodiagnosis of Lyme borreliosis. Med Microbiol Immunol (Berl) 187:213–219
19. Kamradt T, Lengl-Janssen B, Strauss A, Bansal G, Steere AC (1996) Dominant recognition of a *Borrelia burgdorferi* outer surface protein A peptide by T helper cells in patients with treatment-resistant Lyme arthritis. Infect Immun 64:1284–1289
20. Krause A, Brade V, Schoerner C, Solbach W, Kalden JR, Burmester GR (1991) T cell proliferation induced by *Borrelia*

burgdorferi in patients with Lyme borreliosis. Autologous serum required for optimum stimulation. Arthritis Rheum 34:393–402
21. Krause A, Burmester GR, Rensing A, Schoerner C, Schaible UE, Simon MM, Herzer P, Kramer MD, Wallich R (1992) Cellular immune reactivity to recombinant OspA and flagellin from *Borrelia burgdorferi* in patients with Lyme borreliosis. J Clin Invest 90:1077–1084
22. Lengl-Janssen B, Strauss AF, Steere AC, Kamradt T (1994) The T helper cell response in Lyme arthritis: differential recognition of *Borrelia burgdorferi* outer surface protein A in patients with treatment-resistant or treatment-responsive Lyme arthritis. J Exp Med 180:2069–2078
23. Mathiesen MJ, Hansen K, Axelsen N, Halkier-Sorensen L, Theisen M (1996) Analysis of the human antibody response to outer surface protein C of *Borrelia burgdorferi* sensu stricto, *B. garinii* and *B. afzelii*. Med Microbiol Immunol (Berl) 185:121–129
24. Pohl-Koppe A, Wilske B, Weiß M, Schmidt H (1998) Borrelia lymphocytoma in childhood. Pediatr Infect Dis J 17:423–426
25. Pohl-Koppe A, Balashov KE, Steere AC, Logigian EL, Hafler DA (1998) Identification of a T cell subset capable of both IFN-gamma and IL-10 secretion in patients with chronic *Borrelia burgdorferi* infection. J Immunol 160:1804–1810
26. Pohl-Koppe A, Logigian EL, Steere AC, Hafler DA (1999) Cross-reactivity of *Borrelia burgdorferi* and myelin basic protein-specific T cells is not observed in borrelial encephalomyelitis. Cell Immunol 194:118–123
27. Preac-Mursic V, Wilske B, Patsouris E, Jauris S, Will G, Soutschek E, Reinhardt S, Lehnert G, Klockmann U, Mehraein P (1992) Active immunization with pC protein of *Borrelia burgdorferi* protects gerbils against *Borrelia burgdorferi* infection. Infection 20:342–349
28. Roessner K, Fikrig E, Russell JQ, Cooper SM, Flavell RA, Budd RC (1994) Prominent T lymphocyte response to *Borrelia burgdorferi* from peripheral blood of unexposed donors. Eur J Immunol 24:320–324
29. Schaible UE, Gay S, Museteanu C, Kramer MD, Zimmer G, Eichmann K, Museteanu U, Simon MM (1990) Lyme borreliosis in the severe combined immunodeficiency (SCID) mouse manifests predominantly in the joints, heart, and liver. Am J Pathol 137:811–820
30. Schwan TG, Piesman J, Golde WT, Dolan MC, Rosa PA (1995) Induction of an outer surface protein on *Borrelia burgdorferi* during tick feeding. Proc Natl Acad Sci USA 92:2909–2913
31. Simon MM, Bauer Y, Zhong W, Hofmann H, Wallich R (1999) Lyme disease: pathogenesis and vaccine development. Zentralbl Bakteriol Mikrobiol Hyg A 289:690–695
32. Wilske B, Preac-Mursic V (1993) Microbiological diagnosis of Lyme borreliosis. In: Weber K, Burgdorfer W (eds) Aspects of Lyme borreliosis. Springer, Berlin Heidelberg New York, pp 267–300
33. Wilske B, Bader L, Pfister HW, Preac-Mursic V (1991) Diagnostik der Lyme-Neuroborreliose. Fortschr Med 22:441–446
34. Wilske B, Preac-Mursic V, Göbel UB, Graf B, Jauris-Heipke S, Soutschek E, Schwab E, Zumstein G (1993) An OspA serotyping system for *Borrelia burgdorferi* based on reactivity with monoclonal antibodies and OspA sequence analysis. J Clin Microbiol 31:340–350
35. Wilske B, Preac-Mursic V, Jauris S, Hofmann A, Pradel I, Soutschek E, Schwab E, Will G, Wanner G (1993) Immunological and molecular polymorphism of OspC, an immunodominant major outer surface protein of *Borrelia burgdorferi*. Infect Immun 61:2182–2191
36. Wilske B, Fingerle V, Herzer P, Hofmann A, Lehnert G, Peters H, Pfister H-W, Preac-Mursic V, Soutschek E, Weber K (1993) Recombinant immunoblot in the serodiagnosis of Lyme borreliosis. Comparison with indirect immunofluorescence and enzyme-linked immunosorbent assay. Med Microbiol Immunol (Berl) 182:255–270
37. Wilske B, Fingerle D, Preac-Mursic V, Jauris-Heipke S, Hofmann A, Loy H, Pfister H-W, Rössler D, Soutschek E (1994) Immunoblot using recombinant antigens derived from different genospecies of *Borrelia burgdorferi* sensu lato. Med Microbiol Immunol (Berl) 183:43–59
38. Wilske B, Busch U, Fingerle V, Jauris-Heipke S, Preac-Mursic V, Roessler D, Will G (1996) Immunological and molecular variability of OspA and OspC. Implications for Borrelia vaccine development. Infection 24:208–212
39. Wilske B, Busch U, Eiffert H, Fingerle V, Pfister H-W, Roessler D, Preac-Mursic V (1996) Diversity of OspA and OspC among cerebrospinal fluid isolates of *Borrelia burgdorferi* sensu lato from patients with neuroborreliosis in Germany. Med Microbiol Immunol (Berl) 184:195–201
40. Wilske B, Habermann C, Fingerle V, Hillenbrandt B, Jauris-Heipke S, Lehnert G, Pradel I, Rössler D, Schulte-Spechtel U (1999) An improved recombinant IgG immunoblot for serodiagnosis of Lyme borreliosis. Med Microbiol Immunol (Berl) 188:139–144
41. Yoshinari NH, Reinhardt BN, Steere AC (1991) T cell responses to polypeptide fractions of *Borrelia burgdorferi* in patients with Lyme arthritis. Arthritis Rheum 34:707–713
42. Zhong W, Stehle T, Museteanu C, Siebers A, Gern L, Kramer M, Wallich R, Simon MM (1997) Therapeutic passive vaccination against chronic Lyme disease in mice. Proc Natl Acad Sci USA 94:12533–12538
43. Zoschke DC, Skemp AA, Defosse DL (1991) Lymphoproliferative responses to *Borrelia burgdorferi* in Lyme disease. Ann Intern Med 114:285–289