Borrelia miyamotoi Activates Human Dendritic Cells and Elicits T Cell Responses

Mason, Lauren M. K.; Koetsveld, Joris; Trentelman, Jos J. A.; Kaptein, Tanja M.; Hoornstra, Dieuwertje; Wagemakers, Alex; Fikrig, Michelle M.; Ersoz, Jasmin I.; Oei, Anneke; Geijtenbeek, Teunis B. H.; Hovius, Joppe W. R.

Published in:
Journal of immunology (Baltimore, Md.

DOI:
10.4049/jimmunol.1801589

Published: 01/01/2020

Document Version
Peer reviewed version

Citation for pulished version (APA):
Mason, L. M. K., Koetsveld, J., Trentelman, J. J. A., Kaptein, T. M., Hoornstra, D., Wagemakers, A., Fikrig, M. M., Ersoz, J. I., Oei, A., Geijtenbeek, T. B. H., & Hovius, J. W. R. (2020). Borrelia miyamotoi Activates Human Dendritic Cells and Elicits T Cell Responses. *Journal of immunology (Baltimore, Md.*, *204*(2), 386-393. https://doi.org/10.4049/jimmunol.1801589

Borrelia miyamotoi activates human dendritic cells and elicits T cell responses

Lauren M. Mason[a], Joris Koetsveld[a], Alex Wagemakers[a], Michelle M. Fikrig[a], Jasmin I. Ersoz[a], Anneke Oei[b], Teunis B.H. Geijtenbeek[c], Joppe W. Hovius[a,d]

[a] Amsterdam UMC, University of Amsterdam, Center for Experimental and Molecular Medicine, Amsterdam Infection & Immunity, Meibergdreef 9, 1105AZ Amsterdam, Netherlands

[b] Amsterdam UMC, University of Amsterdam, Department of Medical Microbiology, Amsterdam Infection & Immunity, Meibergdreef 9, 1105AZ Amsterdam, Netherlands

[c] Amsterdam UMC, University of Amsterdam, Department of Experimental Immunology, Amsterdam Infection & Immunity, Meibergdreef 9, 1105AZ Amsterdam, Netherlands

[d] Amsterdam UMC, University of Amsterdam, Division of infectious diseases, Meibergdreef 9, 1105AZ Amsterdam, Netherlands

Abstract

The spirochete *Borrelia miyamotoi* has recently been shown to cause relapsing fever. Like the Lyme disease agent, *Borrelia burgdorferi, B. miyamotoi* is transmitted through the bite of infected ticks, however, little is known about the response of the immune system upon infection. Dendritic cells (DCs) play a central role in the early immune response against *B. burgdorferi*. We investigated the response of DCs to two different strains of *B. miyamotoi* using *in vitro* and *ex vivo* models and compared this to the response elicited by *B. burgdorferi.* Our findings show that *B. miyamotoi* is phagocytosed by monocyte derived-DCs, causing upregulation of activation markers and production of proinflammatory cytokines, in a similar manner to *B. burgdorferi.* Recognition of *B. miyamotoi* was demonstrated to be partially mediated by TLR2. DCs migrated out of human skin explants upon inoculation of the skin with *B. miyamotoi.* Finally, we showed that *B. miyamotoi*-stimulated DCs induced proliferation of naïve CD4+ and CD8+ T cells, to a larger extent than *B. burgdorferi.* In conclusion, we show here that DCs respond to and mount a immune response against *B. miyamotoi* that is similar to the response to *B. burgdorferi,* and are able to induce T cell proliferation.

32 **Introduction**

33 The tick-borne relapsing fever spirochete *Borrelia miyamotoi* was recently shown to be
34 pathogenic (1-3). Similar to the Lyme disease agent, *Borrelia burgdorferi* sensu lato, *B.*
35 *miyamotoi* infects *Ixodes* ticks in Europe, North America and Asia, however, the clinical
36 presentation is more similar to other relapsing fever *Borrelia,* as symptoms are more systemic
37 and viral-like and include high fever, headache, myalgia, arthralgia and malaise (3-5). The
38 development of chronic meningoencephalitis has been described in three immunocompromised
39 patients infected with *B. miyamotoi* (1, 2, 6). As yet, it is not clear what the public health burden
40 of this disease is (7). Furthermore, as it has only recently become possible to cultivate *B.*
41 *miyamotoi in vitro* (8), up to now very few *in vitro* studies have been performed and little is
42 known about the immunopathogenesis of *B. miyamotoi*.

43 In contrast, *B. burgdorferi* has been studied extensively *in vitro* and much of the
44 immunopathogenesis has been elucidated. Upon entry into the skin via tick bite, *B. burgdorferi*
45 encounters dendritic cells (DCs); professional antigen presenting cells which orchestrate
46 adaptive immune responses by presenting phagocytosed antigen to CD4+ T cells which they
47 encounter upon migration into draining lymph nodes (9). DCs prime CD4+ T cells to become
48 effector T helper (Th) cells, of which various subsets exist with each a different specialization,
49 depending on the type of response needed to clear the pathogen (10). DCs have been shown to
50 phagocytose and respond to *B. burgdorferi in vitro*, producing cytokines and upregulating
51 activation markers (11). DCs recognize pathogens through pattern recognition receptors (PRR),
52 which can recognize pathogen-associated molecular patterns expressed by pathogens. The

53 central PRR responsible for recognition of *B. burgdorferi* is Toll like receptor 2 (TLR2) (12, 13)
54 that recognizes lipoproteins expressed on the outer surface of the spirochete. We have shown
55 previously that, in an *ex vivo* human skin model, DCs migrated out of the skin in response to
56 inoculation with *B. burgdorferi* in a TLR-2 dependent manner (14). In *in vitro* studies, DCs primed
57 with *B. burgdorferi* were able to induce Th cells, and elicited a protective immune response *in*
58 *vivo* in mice (15). *B. burgdorferi* induced mixed Th1/Th2 responses in mice infected through
59 syringe inoculation (16).

60 It is not known whether *B. miyamotoi* is recognized by DCs and whether the spirochete elicits
61 DC responses. Furthermore, the adaptive T cell responses that arise from DC-*B. miyamotoi*
62 interactions have not been previously studied. In this study, using two laboratory strains of *B.*
63 *miyamotoi* and *in vitro* (monocyte derived (mo)DC) and *ex vivo* (human skin) models, we
64 demonstrated that human DCs phagocytose *B. miyamotoi* and become activated, migrate out of
65 the skin and produce cytokines in response, in a manner similar to *B. burgdorferi*. *B. miyamotoi*-
66 stimulated DCs were able to induce T cell proliferation. Unraveling the ways in which the
67 immune system responds to and fights *B. miyamotoi* is crucial to our understanding of the
68 disease and may help to explain why the clinical presentation of infection with *B. miyamotoi*
69 differs from that of Lyme disease.

70

71 **Materials and methods**

72 Borrelia *culture*

73 *Borrelia miyamotoi* strains HT31 (Asian isolate) and LB-2001 (American isolate) were cultured in
74 MKP-F medium (AMC, the Netherlands) (8) and *Borrelia burgdorferi* sensu stricto strain B31
75 was cultured in MKP medium (AMC, Amsterdam, The Netherlands) at 33°C until cultures
76 reached the mid to late log growth phrase. Spirochetes were assessed for motility, quantified by
77 dark-field microscopy and recovered by centrifugation at 4000 x g for 8 minutes. Subsequently,
78 spirochetes were resuspended in Roswell Park Memorial Institute (RPMI) 1640 medium (Gibco,
79 Paisley, UK) supplemented with 10% foetal calf serum (FCS, Lonza, Verviers, Belgium) and L-
80 glutamine (Gibco) (for *in vitro* experiments) or phosphate buffered saline (PBS) (Fresenius Kabi,
81 Graz, Austria) (for *ex vivo* experiments). Spirochetes were passaged no more than 5 times prior
82 to experimentation.

83 *Monocyte derived dendritic cell (moDC) generation and stimulation*

84 MoDCs were generated from peripheral blood mononuclear cells (PBMCs) from fresh
85 heparinized blood donated by consenting, healthy volunteers as described previously (17, 18). A
86 total of 1 x 10^5 moDCs in 100µl RPMI 1640 medium supplemented with 10% FCS and L-
87 glutamine were stimulated in a 96-wells flat-bottom plate (Corning®, Sigma-Aldrich) for 48
88 hours at 37°C with 5% CO_2 with various multiplicities of infection (MOIs) of *Borrelia* strains, 10
89 ng/ml ultrapure Lipopolysaccharide (LPS) of *Escherichia coli* (Invitrogen, Paisley, UK) or 50 ng/ml
90 Pam3CSK4 (Invivogen, San Diego, CA, USA). For lysate stimulation experiments, lysates of LB-
91 2001 strains expressing vsp1 or vlpC2 were prepared as previously reported (19). In selected
92 experiments 10 µg/ml TLR2-blocking αTLR2.5 (Ebioscience, San Diego, CA, USA) was used. Cells
93 were washed and resuspended in FACS buffer (0.5% bovine serum albumin (BSA), 0.01% NaN_3

94 and 0.35 mM EDTA in PBS) and stained with fluorophore-conjugated antibodies against HLA-DR,
95 CD83 or CD86 (BD Biosciences, Franklin Lakes, NJ) and flow cytometry was performed using a
96 FACS-CANTO II (BD Biosciences). The results were analysed using Flow-Jo (Treestar, Ashland,
97 OR) and Prism 7 (GraphPad Software, La Jolla, CA).

98 *Phagocytosis*

99 Phagocytosis experiments were performed in essence as previously described (17). Viable CFSE-
100 stained *B. burgdorferi or B. miyamotoi* were incubated with moDC at 37°C at an MOI of 5 or 50
101 for various time points. Phagocytosis was stopped by adding ice-cold FACS buffer (0.5% bovine
102 serum albumin (BSA), 0.01% NaN_3 and 0,35 mM EDTA in PBS) and putting the samples at 4°C.
103 The cells were then washed and analysed with flow cytometry. The phagocytosis index was
104 defined as the mean fluorescence intensity × fraction CFSE-positive cells, minus the 4 ◦C
105 binding control.

106 *Lymphocyte proliferation*

107 PBLs were isolated from fresh heparinised blood donated by consenting, healthy volunteers by
108 density centrifugation on Ficoll and Percoll (both GE healthcare, Chicago, IL), washed in PBS and
109 labelled with 1 µM CFSE in PBS. Labelled PBLs were then co-cultured together with washed
110 allogenic *Borrelia*-stimulated moDCs at DC:PBL ratios of 1:1, 1:2 and 1:5 in 96-wells flat bottom
111 plates (Corning®, Sigma-Aldrich) in Iscove's Modified Dulbecco's Medium (IMDM, Gibco) with
112 10% FCS. After four days, supernatant was harvested and PBLs were stained with fluorophore-
113 conjugated αCD4 and αCD8 antibodies (BD Biosciences) and analyzed by flow cytometry. For

114 each DC:PBL donor combination, the DC:PBL ratio which produced optimal proliferation results
115 was selected and is shown in the results and analysis. *Borrelia*-stimulated moDCs were
116 confirmed to have similar expression of maturation markers and cytokine production to earlier
117 results prior to co-culture. All donors were seronegative, as measured by a commercially
118 available *B. burgdorferi* C6 ELISA (Immunectis, XX, USA).

119 *Ex vivo experiments*

120 *Ex vivo* skin experiments were conducted as previously described (14, 18). Briefly, human
121 surplus skin from breast or abdomen was obtained from healthy donors undergoing corrective
122 surgery at the Kennemer Gasthuis Haarlem, the Netherlands. A total of 10^4 or 10^6 spirochetes or
123 100 ng/ml ultrapure LPS of *Escherichia coli* were injected intradermally in 50 ul PBS. Eight mm
124 biopsies were taken immediately from the injection site and then washed in Iscove's Modified
125 Dulbecco's Medium (IMDM) (Life Technologies, Paisley, UK) with 2% FCS, before being
126 transferred into 1 ml IMDM with 10% FCS and L-glutamine - without antibiotics - and incubated
127 for 48 hours at 37°C with 5% CO_2. Cells were harvested, washed twice, counted and
128 resuspended in FACS buffer for staining with fluorophore-conjugated antibodies against HLA-
129 DR, CD11c, CD83 and CD1a (BD Biosciences). Flow cytometry was performed as for moDCs. The
130 number of DCs migrated per condition was calculated from the percentage of total migrated
131 cells that were HLA-DR+/CD11c+. The supernatant and migrated cells from three separate
132 biopsies, taken from random locations of the skin, were pooled together to constitute one
133 replicate of each condition.

134 *Cytokine measurement*

135 Concentrations of the cytokines IL-1β, IL-6, IL-8, TNFα, IL-12p70 and IL-10 were measured in
136 supernatant of 48h stimulated moDCs (1 x 10^5 in 100µl) or the supernatant of migrated DCs
137 using a human inflammatory cytometric bead array (CBA) kit (BD Biosciences), according to the
138 manufacturer's instructions. Results were analyzed using Flow-Jo and GraphPad Prism 7
139 software.

140 *Statistics*

141 Statistical analysis was performed with GraphPad Prism 7. Data was considered significant at $p <$
142 0.05, with one asterisk (*) representing $0.01 < p < 0.05$, two asterisks (**) representing $0.001 < p$
143 < 0.01 and three asterisks (***) representing $0.0001 < p < 0.001$. One-way ANOVA and Tukey's
144 post-hoc tests were used to analyze normally distributed data and Kruskal-Wallis and Dunn's
145 post-hoc tests were used to analyse data that was not normally distributed.

146 *Ethics statement*

147 *In vitro* experiments were performed using surplus human healthy volunteer blood collected for
148 the purpose of a separate study. Written informed consent was obtained from human blood
149 donors in accordance with protocols approved by the Medical Ethics Committee of the
150 Academic Medical Center (Approval number NL34294.018.10) and the declaration of Helsinki.
151 As for the human skin, the Medical Ethical Committee of the Academic Medical Center,
152 Amsterdam, deemed that no approval was necessary for the use of (otherwise) discarded
153 tissue.

154

155 **Results**

156 ***B. miyamotoi* is phagocytosed by DCs**

157 To determine whether DCs are capable of phagocytosing *B. miyamotoi,* monocyte-derived DCs
158 were incubated with CFSE-labelled strains HT31 and LB-2001 for various time points at 37°C.
159 Uptake by DCs was analyzed by flow cytometry and compared to that of *B. burgdorferi* strain
160 B31. The CFSE signal (fig 1a) and phagocytosis index (fig 1b) of DCs incubated with both *B.*
161 *miyamotoi* strains increased over time, as similarly observed for B31, indicating that *B.*
162 *miyamotoi* is phagocytosed by DCs, in a comparable manner to *B. burgdorferi*.

163 ***B. miyamotoi* activates DCs to produce cytokines**

164 The activation of DCs and production of cytokines are crucial for their function. DCs were
165 stimulated with viable *B. miyamotoi* strains HT31 and LB-2001 and *B. burgdorferi* strain B31 for
166 48 hours at an MOI of 5 and 50 and maturation marker expression was measured by flow
167 cytometry (fig 2a,b). Notably, expression of the maturation markers CD83 and CD86 was
168 significantly upregulated by stimulation with *B. miyamotoi* strains at both MOIs, compared to
169 unstimulated DCs, similarly to stimulation with LPS. The *B. burgdorferi* strain B31 induced CD83
170 expression at both MOIs, whereas CD86 was only significantly upregulated at the higher MOI.
171 HLA-DR expression was only significantly induced by HT31 at an MOI of 50. Next we measured
172 cytokine production by *B. miyamotoi*-stimulated DCs (fig 2c). Both *B. miyamotoi* strains as well
173 as *B. burgdorferi* strain B31 induced IL-8, IL-6 and TNFα production at an MOI of 50. DCs
174 stimulated with *B. miyamotoi* strains also produced IL-12, at significantly higher levels than B31-
175 stimulated DCs, and HT31-stimulated DCs additionally produced significantly higher levels of IL-

176 1β than unstimulated DCs. These results indicate that *B. miyamotoi* strains are capable of
177 inducing DC activation and cytokine production in a similar manner to *B. burgdorferi.*

178 *B. miyamotoi* expresses variable major proteins (VMPs) – surface lipoproteins that form the
179 basis of the humoral immune response against the spirochete, and populations of spirochetes
180 have different VMP expression by spontaneous full gene switching in an attempt to evade
181 antibody responses. In a prelimary effort to explore whether VMPs affect the activation of DCs
182 by *B. miyamotoi* strains, we measured the maturation markers of DCs stimulated with lysates of
183 LB-2001 containing different VMPs. We selected an LB-2001 that expressed the surface
184 lipoprotein variable small protein 1 (Vsp1), an LB-2001 strain that had switched to express
185 variable large protein C2 (VlpC2) after challenge with αVsp1 antibodies, and an LB-2001 strain
186 challenged with αOVA (i.e. still expressing Vsp1) as a control. Our preliminary data show that all
187 three lysates (at a concentration of 10 ng/ml) induced upregulation of CD83 and CD86 and no
188 differences were observed in the expression of these markers between the different lysates,
189 suggesting that activation of DCs by *B. miyamotoi* is independent of the VMP expressed (data
190 not shown).

191 **DCs migrate out of the skin in response to inoculation of *B. miyamotoi***

192 To investigate whether *B. miyamotoi* activates DCs in a more physiological *ex vivo* model, we
193 inoculated human skin with HT31 or LB-2001, took biopsies of the inoculated skin and cultured
194 the biopsies in medium for 48 hours, and we analysed the number of migrated DCs. Migration
195 of DCs from skin inoculated with HT31 or LB-2001 was approximately 10-fold higher than
196 migration of DCs from PBS-inoculated skin, and similar to (i.e. not statistically different from)

197 the numbers of DCs migrated from B31-inoculated skin (fig 3a). Preliminary data indicate that,
198 as we have previously shown for *B. burgdorferi* [REF], that LCs consisted of the minory of
199 migrated cells upon *B. miyamotoi* inoculation, i.e. 0,5-2% (data not shown). In addition,
200 significantly higher levels of the proinflammatory cytokines IL-6 and IL-8, but not IL-1β or IL-10
201 were measured in the supernatants of *Borrelia*-inoculated biopsies compared to PBS-inoculated
202 biopsies (fig 3b). IL-12 and TNFα levels were under the detection limit. These results strongly
203 suggest that *B. miyamotoi* induces DC migration from the skin.

204 **DC responses to *B. miyamotoi* are partially mediated by TLR2**

205 To determine whether TLR2, the main PRR responsible for recognition of *B. burgdorferi,* is
206 involved in the recognition of *B. miyamotoi,* DCs were preincubated with or without a blocking
207 TLR2 antibody and stimulated with HT31 or LB-2001 for 48 hours. Notably, the antibody against
208 TLR2 strongly diminished the expression of CD83 induced by both strains (fig 4b). Moreover, the
209 TLR2 antibody suppressed CD83 upregulation induced by TLR2-agonist Pam3CSK4, but not that
210 induced by the TLR4-agonist LPS, indicating that the antibody is specific for TLR2 (fig 4a). CD86
211 upregulation in response to HT31 was also abrogated by the TLR2 antibody, and a trend was
212 observed towards lower CD86 expression in LB-2001 stimulated DCs, although this was not
213 significant. A trend toward lower production of the cytokines IL-12 and IL-6 (fig 4b) as well as IL-
214 8 and TNFα (data not shown), by *B. miyamotoi*-stimulated DCs preincubated with αTLR2 was
215 also observed, although not statistically significant. These results demonstrate that TLR2 plays a
216 role in DC recognition of *B. miyamotoi.*

217 ***B. miyamotoi*–stimulated DCs are better inducers of PBL proliferation than *B. burgdorferi*-**
218 **stimulated DCs**

219 In order to explore the adaptive responses induced by *B. miyamotoi*-stimulated DCs, and
220 compare these to DCs stimulated by *B. burgdorferi,* DCs stimulated with HT31, LB-2001 or B31
221 for 48 hours were co-cultured with allogenic CFSE-labelled PBLs for 4 days, after which time the
222 CFSE signal of responder cells was measured by flow cytometry. Proliferating cells had a lower
223 CFSE signal, as CFSE was halved between daughter cells during cell division (fig 5a). Significantly
224 higher percentages of total PBLs, CD4+ and CD8+ T cells were induced to proliferate by HT31-
225 and LB-2001-stimulated DCs compared to unstimulated DCs (fig 5b,c,d). The percentage of
226 proliferating PBLs induced by *B. miyamotoi*-stimulated DCs was also significantly higher than
227 that of *B. burgdorferi*-stimulated DCs, which did not induce significant proliferation compared to
228 unstimulated DCs. These results demonstrate that DCs stimulated by *B. miyamotoi* are able to
229 induce T cell proliferation.

230

231 **Discussion**

232 In this study, we investigated the response of DCs to *B. miyamotoi*, from recognition to
233 induction of T cell responses. Uptake of pathogens and activation of DCs is the first step towards
234 orchestration of adaptive immune responses. We demonstrated that DCs were able to
235 phagocytose two different strains of *B. miyamotoi,* HT31 and LB-2001, in a manner similar to the
236 *B. burgdorferi* strain B31. Most probably due to technical reason we were unable to show

237 phagocytosis in the ex vivo skin model (data not shown). After 48 hours of stimulation, DCs
238 became activated, upregulating the costimulatory molecule CD86, necessary for T cell
239 activation, and CD83 which characterizes DC activation. HLA-DR, the major histocompatibility
240 complex II molecule which presents antigen to T cells was also upregulated in several
241 experiments although this varied among donors. Proinflammatory cytokines IL-6, IL-8 and TNFα
242 were produced by DCs in response to *B. miyamotoi* strains at high MOIs, as with B31. *B.*
243 *miyamotoi* strains elicited a greater cytokine response than *B. burgdorferi,* as, unlike stimulation
244 with B31, DCs stimulated with HT31 or LB-2001 additionally produced the Th1-inducing cytokine
245 IL-12 and HT31 induced production of significantly higher concentrations of IL-1β, although a
246 trend was also seen for LB-2001 and B31 and which may be significant at higher MOIs.

247 *B. miyamotoi* induced migration of DCs out of human skin explants in a more physiological *ex*
248 *vivo* model of early *B. miyamotoi* infection. This result was comparable to previous work in
249 which we have shown that DCs migrate out of skin in response to the spirochetes *B. burgdorferi*
250 (14), *Treponema pallidum* and *Leptospira interrogans* (18). Spirochetes were inoculated
251 intradermally in numbers comparable to those found in biopsies of erythema migrans, in order
252 to mimic a tick bite (20). Due to variations in the skin used for experiments, i.e. thickness,
253 region (breast or abdomen), age of donor we observed variability in the migration of DCs in
254 response to stimulation. This variation is in line with our previous observations. As expected,
255 no differences in the expression of activation markers was observed in DCs migrated from *B.*
256 *miyamotoi* and PBS inoculated skin (data not shown) as DCs that have migrated – also in
257 response to PBS – have a mature and activated phenotype. However, skin explants inoculated

258 with *B. miyamotoi* produced greater amounts of IL-6 and IL-8, whereas IL-1β and IL-10
259 production was not significantly induced after inoculation by either *B. miyamotoi* or *B.*
260 *burgdorferi* compared to PBS, as we have observed in previous experiments with *B. burgdorferi*
261 (14). Although it is likely that DCs are the main producers of these cytokines, alongside DCs,
262 other possible sources for the cytokine production observed in inoculated skin are keratinocytes
263 and fibroblasts (21, 22).

264 Our data strongly suggest that TLR-2 is involved in the recognition of *B. miyamotoi* by DCs, as
265 blocking the receptor using an antagonistic TLR2 antibody impaired activation and cytokine
266 production in response to *B. miyamotoi* stimulation. TLR2 recognises diacylated and triacylated
267 lipopeptides by forming heterodimers with TLR1 and TLR6 (23, 24). Upon recognition, the
268 Myeloid differentiation primary response 88-dependent activation of nuclear factor κB signaling
269 cascade is induced, which triggers transcription of co-stimulatory molecules required for T cell
270 activation, pro-inflammatory and anti-inflammatory cytokines, matrix metalloproteinases and
271 adhesion molecules, which enable the maturation and migration of DCs (9). We did not observe
272 a complete block of *B. miyamotoi*-induced DC activation and cytokine production upon blocking
273 TLR2. This may be due to incomplete blocking by the antagonistic antibody, as we also observed
274 residual cytokine production by DCs stimulated by the TLR2 agonist Pam3CSK4 in the presence
275 of aTLR2. Another explanation could be that other PRR are involved in the recognition of *B.*
276 *miyamotoi* alongside TLR2. Since other PRRs such as TLR5, 7,8 and 9 have been reported to
277 recognize *B. burgdorferi* (9), they may be similarly implicated in recognition of *B. miyamotoi*.

278 As with other tick-borne relapsing fever spirochetes, *B. miyamotoi* expresses variable major
279 proteins (VMPs) including Vlps and Vsps (25, 26), surface proteins that are the basis of the
280 humoral immune response against relapsing fever spirochetes. The *B. miyamotoi* strain LB-2001
281 has recently been demonstrated to predominantly express Vsp1, with a minority population
282 expressing VlpC2. After infection of a mammalian host, an IgM response is mounted against the
283 predominantly expressed VMP, in this case Vsp1, however the minority populations expressing
284 a different VMP are not affected and are able to evade the antibody response of the host,
285 enabling relapses in spirochetemia to occur (19). Our preliminary data suggest that DCs become
286 activated in response to LB-2001 strains expressing either VMP, indicating that they are capable
287 of recognizing either or that other spirochetal proteins are driving the activation.

288 DCs stimulated with *B. miyamotoi* were able to elicit a T cell response, as we demonstrated
289 proliferation of the CD4+ and CD8+ PBL fractions, as well as total PBLs using an allogenic MLR.
290 This response was greater than that generated by *B. burgdorferi*-stimulated DCs. The reason
291 remains to be investigated. Regardless, differences in DC-induced adaptive immune responses
292 might explain why *B. miyamotoi* appears to be readily cleared after human infection - and
293 relapses only seldomly occur - and *B. burgdorferi* is able to cause an chronic infection. Indeed,
294 preliminary unpublished data indicate that *B. burgdorferi* was able to inhibit expression of genes
295 involved in antigen presentation in monocytes to a greater extent than *B. miyamotoi* (personal
296 communication Leo Joosten, Radboudumc). *B. miyamotoi*-stimulated DCs released IL-12, a Th1
297 polarizing cytokine. Of interest, Th1 and Th2 responses have previously been implicated in *B.*
298 *burgdorferi* infection; IL-4 and IFN-γ were detected in the lymph nodes of mice infected with *B.*

299 *burgdorferi* (16), and Th1 responses have been observed in the joints and skin of Lyme
300 borreliosis patients (27-29). Further studies should determine which subsets of Th cells are
301 primed as part of the immune response to *B. miyamotoi.*

302 In summary, we have shown that *B. miyamotoi* strains HT31 and LB-2001 are phagocytosed by,
303 and activate DCs, leading to upregulation of activation markers and production of
304 proinflammatory cytokines in a manner similar to that of the *B. burgdorferi* strain B31. In
305 addition, *B. miyamotoi* activated DCs to migrate out of human skin explants. Activation of DCs
306 was found only to be partially dependent on TLR2. Interestingly, *B. miyamotoi*-stimulated DCs
307 induced significantly more proliferation of CD4+ and/or CD8+ T cells compared to *B. burgdorferi*-
308 stimulated DCs.

309

310 **Acknowledgements**

311 We are very grateful to Wouter J. van der Pot, K. Ahmed and J.G. van Eck, Department of Plastic
312 Surgery, Kennemer Gasthuis Haarlem, for providing us with skin for this study. Furthermore, we
313 would like to thank C. van 't Veer, Center for Experimental and Molecular Medicine, Academic
314 Medical Center, Amsterdam, J.K. Sprokholt and T.M. Kaptein, department of Experimental
315 Immunology, Academic Medical Center, Amsterdam, for their invaluable help and advice with
316 assays. This study was supported by ZonMW as part of the project 'Ticking on Pandora's Box, a
317 study into tick-borne pathogens in Europe' (project no. 50-52200-98-313) and TBHG was
318 supported by ERC (Sentinel grant 670424).

319

320 **References**

321 1. Gugliotta, J. L., H. K. Goethert, V. P. Berardi, and S. R. Telford, 3rd. 2013. Meningoencephalitis
322 from Borrelia miyamotoi in an immunocompromised patient. *N Engl J Med* 368: 240-245.
323 2. Hovius, J. W., B. de Wever, M. Sohne, M. C. Brouwer, J. Coumou, A. Wagemakers, A. Oei, H. Knol,
324 S. Narasimhan, C. J. Hodiamont, S. Jahfari, S. T. Pals, H. M. Horlings, E. Fikrig, H. Sprong, and M.
325 H. van Oers. 2013. A case of meningoencephalitis by the relapsing fever spirochaete Borrelia
326 miyamotoi in Europe. *Lancet* 382: 658.
327 3. Platonov, A. E., L. S. Karan, N. M. Kolyasnikova, N. A. Makhneva, M. G. Toporkova, V. V. Maleev,
328 D. Fish, and P. J. Krause. 2011. Humans infected with relapsing fever spirochete Borrelia
329 miyamotoi, Russia. *Emerg Infect Dis* 17: 1816-1823.
330 4. Krause, P. J., S. Narasimhan, G. P. Wormser, L. Rollend, E. Fikrig, T. Lepore, A. Barbour, and D.
331 Fish. 2013. Human Borrelia miyamotoi infection in the United States. *N Engl J Med* 368: 291-293.
332 5. Molloy, P. J., S. R. Telford, 3rd, H. R. Chowdri, T. J. Lepore, J. L. Gugliotta, K. E. Weeks, M. E.
333 Hewins, H. K. Goethert, and V. P. Berardi. 2015. Borrelia miyamotoi Disease in the Northeastern
334 United States: A Case Series. *Ann Intern Med* 163: 91-98.
335 6. Boden, K., S. Lobenstein, B. Hermann, G. Margos, and V. Fingerle. 2016. Borrelia miyamotoi-
336 Associated Neuroborreliosis in Immunocompromised Person. *Emerg Infect Dis* 22: 1617-1620.
337 7. Wagemakers, A., P. J. Staarink, H. Sprong, and J. W. Hovius. 2015. Borrelia miyamotoi: a
338 widespread tick-borne relapsing fever spirochete. *Trends Parasitol* 31: 260-269.
339 8. Wagemakers, A., A. Oei, M. M. Fikrig, W. R. Miellet, and J. W. Hovius. 2014. The relapsing fever
340 spirochete Borrelia miyamotoi is cultivable in a modified Kelly-Pettenkofer medium, and is
341 resistant to human complement. *Parasit Vectors* 7: 418.
342 9. Mason, L. M., C. C. Veerman, T. B. Geijtenbeek, and J. W. Hovius. 2014. Menage a trois: Borrelia,
343 dendritic cells, and tick saliva interactions. *Trends Parasitol* 30: 95-103.
344 10. de Jong, E. C., H. H. Smits, and M. L. Kapsenberg. 2005. Dendritic cell-mediated T cell
345 polarization. *Springer Semin Immunopathol* 26: 289-307.
346 11. Suhonen, J., J. Komi, J. Soukka, O. Lassila, and M. K. Viljanen. 2003. Interaction between Borrelia
347 burgdorferi and immature human dendritic cells. *Scand J Immunol* 58: 67-75.
348 12. Hirschfeld, M., C. J. Kirschning, R. Schwandner, H. Wesche, J. H. Weis, R. M. Wooten, and J. J.
349 Weis. 1999. Cutting edge: inflammatory signaling by Borrelia burgdorferi lipoproteins is
350 mediated by toll-like receptor 2. *J Immunol* 163: 2382-2386.
351 13. Shin, O. S., R. R. Isberg, S. Akira, S. Uematsu, A. K. Behera, and L. T. Hu. 2008. Distinct roles for
352 MyD88 and Toll-like receptors 2, 5, and 9 in phagocytosis of Borrelia burgdorferi and cytokine
353 induction. *Infect Immun* 76: 2341-2351.
354 14. Mason, L. M., A. Wagemakers, C. van 't Veer, A. Oei, W. J. van der Pot, K. Ahmed, T. van der Poll,
355 T. B. Geijtenbeek, and J. W. Hovius. 2016. Borrelia burgdorferi Induces TLR2-Mediated Migration
356 of Activated Dendritic Cells in an Ex Vivo Human Skin Model. *PLoS One* 11: e0164040.
357 15. Mbow, M. L., N. Zeidner, N. Panella, R. G. Titus, and J. Piesman. 1997. Borrelia burgdorferi-pulsed
358 dendritic cells induce a protective immune response against tick-transmitted spirochetes. *Infect*
359 *Immun* 65: 3386-3390.
360 16. Christe, M., B. Rutti, and M. Brossard. 2000. Cytokines (IL-4 and IFN-gamma) and antibodies (IgE
361 and IgG2a) produced in mice infected with Borrelia burgdorferi sensu stricto via nymphs of
362 Ixodes ricinus ticks or syringe inoculations. *Parasitol Res* 86: 491-496.

363 17. Mason, L. M., E. A. Herkes, M. A. Krupna-Gaylord, A. Oei, T. van der Poll, G. P. Wormser, I.
364 Schwartz, M. M. Petzke, and J. W. Hovius. 2015. Borrelia burgdorferi clinical isolates induce
365 human innate immune responses that are not dependent on genotype. *Immunobiology* 220:
366 1141-1150.
367 18. Mason, L. M. K., and J. W. R. Hovius. 2018. Investigating Human Dendritic Cell Immune
368 Responses to Borrelia burgdorferi. *Methods Mol Biol* 1690: 291-299.
369 19. Wagemakers, A., J. Koetsveld, S. Narasimhan, M. Wickel, K. Deponte, B. Bleijlevens, S. Jahfari, H.
370 Sprong, L. S. Karan, D. S. Sarksyan, T. van der Poll, L. K. Bockenstedt, A. D. Bins, A. E. Platonov, E.
371 Fikrig, and J. W. Hovius. 2016. Variable Major Proteins as Targets for Specific Antibodies against
372 Borrelia miyamotoi. *J Immunol* 196: 4185-4195.
373 20. Liveris, D., G. Wang, G. Girao, D. W. Byrne, J. Nowakowski, D. McKenna, R. Nadelman, G. P.
374 Wormser, and I. Schwartz. 2002. Quantitative detection of Borrelia burgdorferi in 2-millimeter
375 skin samples of erythema migrans lesions: correlation of results with clinical and laboratory
376 findings. *J Clin Microbiol* 40: 1249-1253.
377 21. Marchal, C. M., B. J. Luft, X. Yang, J. Sibilia, B. Jaulhac, and N. M. Boulanger. 2009. Defensin is
378 suppressed by tick salivary gland extract during the in vitro interaction of resident skin cells with
379 Borrelia burgdorferi. *J Invest Dermatol* 129: 2515-2517.
380 22. Schramm, F., A. Kern, C. Barthel, S. Nadaud, N. Meyer, B. Jaulhac, and N. Boulanger. 2012.
381 Microarray analyses of inflammation response of human dermal fibroblasts to different strains of
382 Borrelia burgdorferi sensu stricto. *PLoS One* 7: e40046.
383 23. Takeuchi, O., T. Kawai, P. F. Muhlradt, M. Morr, J. D. Radolf, A. Zychlinsky, K. Takeda, and S.
384 Akira. 2001. Discrimination of bacterial lipoproteins by Toll-like receptor 6. *Int Immunol* 13: 933-
385 940.
386 24. Takeuchi, O., S. Sato, T. Horiuchi, K. Hoshino, K. Takeda, Z. Dong, R. L. Modlin, and S. Akira. 2002.
387 Cutting edge: role of Toll-like receptor 1 in mediating immune response to microbial
388 lipoproteins. *J Immunol* 169: 10-14.
389 25. Barbour, A. G. 2016. Multiple and Diverse vsp and vlp Sequences in Borrelia miyamotoi, a Hard
390 Tick-Borne Zoonotic Pathogen. *PLoS One* 11: e0146283.
391 26. Hamase, A., Y. Takahashi, K. Nohgi, and M. Fukunaga. 1996. Homology of variable major protein
392 genes between Borrelia hermsii and Borrelia miyamotoi. *FEMS Microbiol Lett* 140: 131-137.
393 27. Gross, D. M., A. C. Steere, and B. T. Huber. 1998. T helper 1 response is dominant and localized
394 to the synovial fluid in patients with Lyme arthritis. *J Immunol* 160: 1022-1028.
395 28. Salazar, J. C., C. D. Pope, T. J. Sellati, H. M. Feder, Jr., T. G. Kiely, K. R. Dardick, R. L. Buckman, M.
396 W. Moore, M. J. Caimano, J. G. Pope, P. J. Krause, J. D. Radolf, and N. Lyme Disease. 2003.
397 Coevolution of markers of innate and adaptive immunity in skin and peripheral blood of patients
398 with erythema migrans. *J Immunol* 171: 2660-2670.
399 29. Yssel, H., M. C. Shanafelt, C. Soderberg, P. V. Schneider, J. Anzola, and G. Peltz. 1991. Borrelia
400 burgdorferi activates a T helper type 1-like T cell subset in Lyme arthritis. *J Exp Med* 174: 593-
401 601.

402

403 **Figure legends**

404 **Fig. 1: DCs phagocytose *B. miyamotoi***

405 MoDCs were incubated with CFSE-stained *Borrelia* strains for various time points. a) The CFSE
406 positive DC gate was determined based on the CFSE expression of a positive control of DCs
407 incubated with mixed *Borrelia* compared to DCs alone (left histogram). The CFSE MFI of DCs
408 increased over time for moDCs incubated with all three *Borrelia* strains at an MOI of 50 (right
409 three histograms). b) The phagocytosis index ((% CFSE+ DCs x MFI at 37°C) minus (% CFSE+ DCs x
410 MFI at 4°C)) increased over time for all three *Borrelia* strains, at an MOI of 5 and 50. Graphs
411 show one representative experiment of two independent experiments.

412 **Fig. 2: DCs become mature and produce cytokines in response to *B. miyamotoi***

413 MoDCs were stimulated with *Borrelia* strains for 48 hours. a) The MFI of maturation markers
414 CD83, CD86 and HLA-DR increased on DCs stimulated with each of the three *Borrelia* strains at
415 an MOI of 5 and 50 compared to unstimulated DCs. Histograms are from one respresentative
416 experiment of eight. b) Fold difference in MFI of CD83, CD86 and HLA-DR compared to the
417 unstimulated control increased on DCs stimulated with each *Borrelia* strain. Graphs show the
418 mean (SEM) of eight independent experiments and donors. Asterisks denote significance of
419 difference compared to unstimulated control. c) DCs stimulated with B31, HT31 or LB-2001 at
420 an MOI of 50 produced higher amounts of IL-8, IL-6 and TNFα than unstimulated DCs. In
421 addition, moDCs stimulated with the *B. miyamotoi* isolates HT31 and LB-2001 produced IL-12,
422 at significantly higher levels than B31-stimulated moDCs. HT31-stimulated moDCs additionally
423 secreted IL-1β. Graphs show the mean (SEM) of 8 independent experiments and donors.
424 Asterisks denote significance of differences compared to unstimulated control.

425 **Fig. 3: Skin DCs migrate out of the skin in response to inoculation with *B. miyamotoi***

426 Human skin was inoculated with *Borrelia* strains. a) Inoculation of HT31 or LB-2001 spirochetes
427 induced migration of DCs out of the skin comparable to B31 and LPS injection and significantly
428 greater than PBS injection. b) Skin injected with HT31 or LB-2001 produced significantly more IL-
429 6 and IL-8 but not IL-1β or IL-10 than PBS-injected skin, as measured in the supernatant of
430 biopsies. Graphs show one representative experiment of two independent experiments. Bars
431 represent the SEM of triplicate measurements; each triplicate consists of three pooled biopsies.
432 Asterisks denote significance of differences compared to PBS-injected skin.

433 **Fig. 4: Blocking TLR2 partially supresses DC responses to B. miyamotoi**

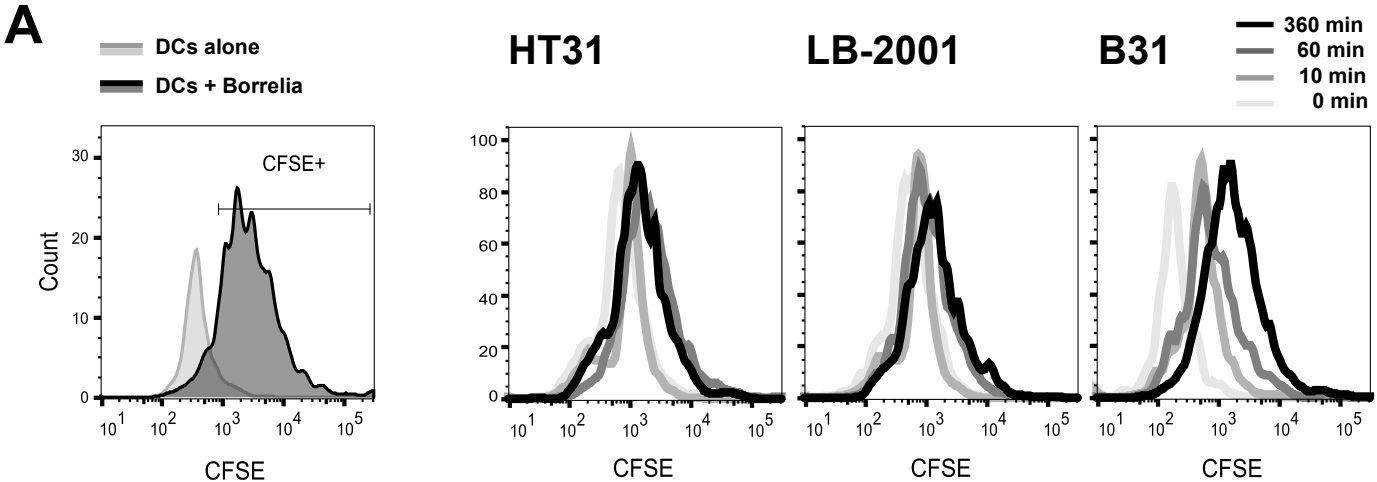
434 MoDCs were incubated with a blocking αTLR2 antibody prior to stimulation with *B. miyamotoi.*
435 a) The increase in CD83 expression in response to HT31 or LB-2001 stimulation at an MOI of 5 or
436 50, and the increase in CD86 expression in response to HT31 stimulation, were impaired by
437 preincubation with αTLR2. The response to LPS was unaffected, whereas CD83 upregulation by
438 Pam3CSK4 (Pam) was abrogated by blocking TLR2. b) A trend was observed toward lower

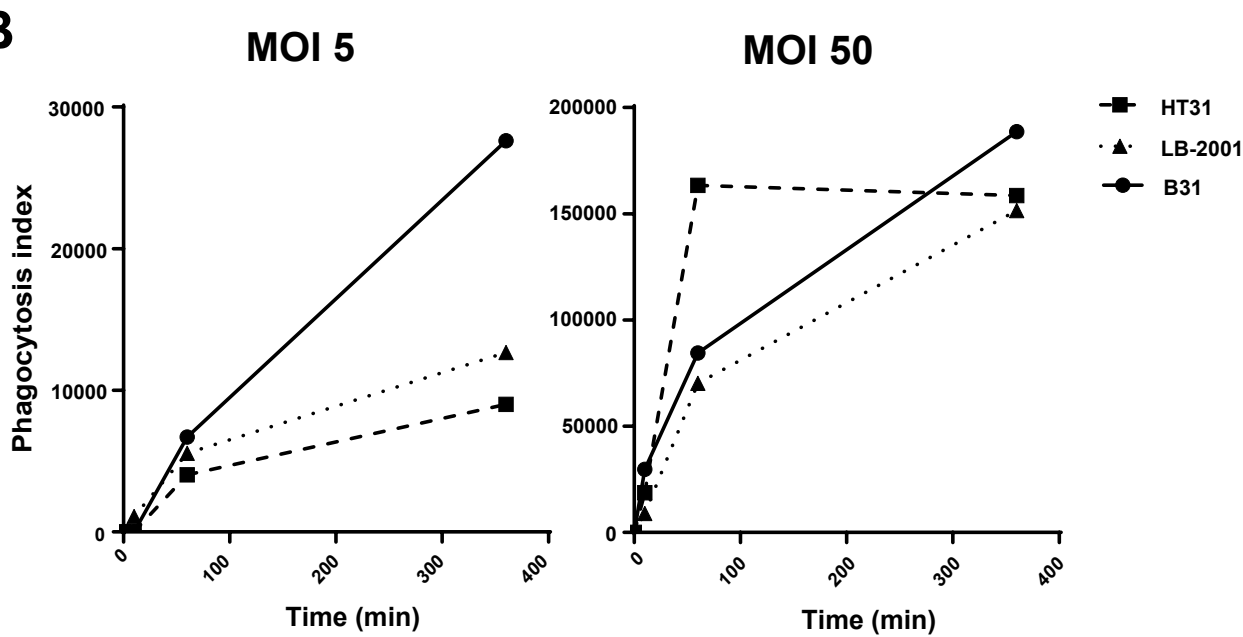
439 production of cytokines IL-12 and IL-6, as well as TNFα and IL-8 (not shown), in response to
440 stimulation after preincubation with αTLR2. Graphs show one representative experiment of
441 three independent experiments. Error bars represent SEM of triplicate measurements. Asterisks
442 denote significant differences between stimulated moDCs with and without blocking TLR2, for
443 each condition.

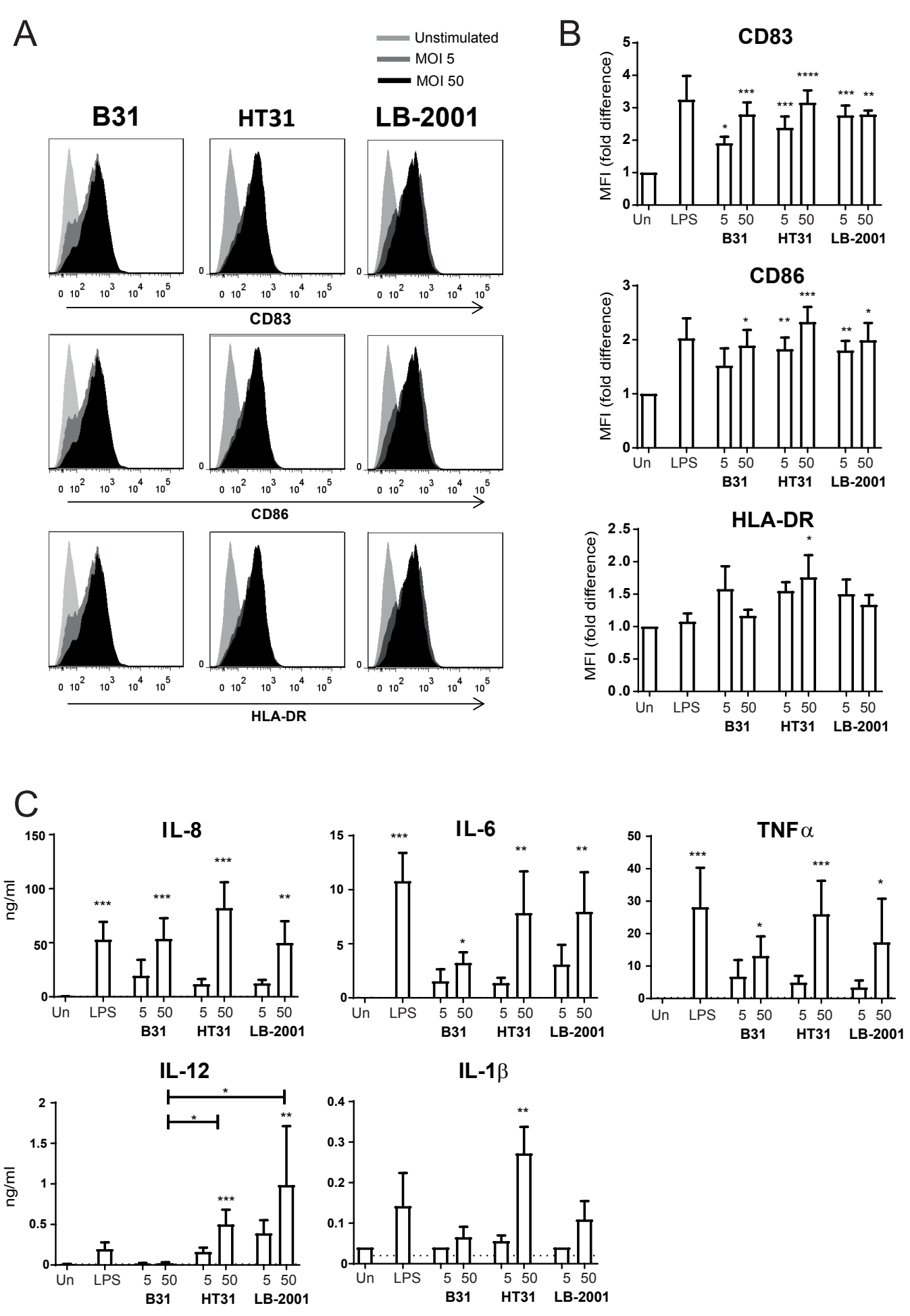
444 **Fig 5: *B. miyamotoi*–stimulated DCs induce greater proliferation of PBLs than *B. burgdorferi*-**
445 **stimulated DCs**

446 DCs stimulated with B31, HT31 and LB-2001 at an MOI of 50 for 48 hours were co-cultured with
447 allogenic CFSE-stained PBLs for 4 days. a) The lower CFSE signal peak in proliferating PBLs
448 stimulated with αCD28 and αCD3 compared to PBLs alone was used to determine the gate for
449 proliferating cells. b) Higher percentages of PBLs proliferated in response to HT31- and LB-2001-
450 stimulated DCs than B31-stimulated DCs. c) CD4+ T cells had a lower CFSE signal after PBL co-
451 culture with HT31- and LB-2001-stimulated DCs. This was less pronounced after co-culture with
452 B31-stimulated DCs. d) Pooled results of four experiments using different DC:PBL donor
453 combinations revealed that a significantly higher percentage of PBLs were induced to proliferate
454 by HT31- and LB-2001-stimulated DCs compared to unstimulated DCs. The percentage of
455 proliferating PBLs induced by B31-stimulated DCs was not significantly different to
456 unstimulated. This was also true for the CD4+ and CD8+ T cell populations of PBLs. Furthermore,
457 HT31- and LB-2001 stimulated DCs induced significantly higher percentages of proliferating total
458 PBLs and CD8+ T cells compared to B31-stimulated DCs and HT31-stimulated DCs induced a
459 significantly higher percentage of proliferating CD4+ T cells than B31-stimulated DCs. Graphs
460 show the mean (SEM) of pooled results of the best DC:PBL ratio of each of four independent
461 DC:PBL donor combinations. Asterisks denote significance of difference compared to
462 unstimulated control DCs.

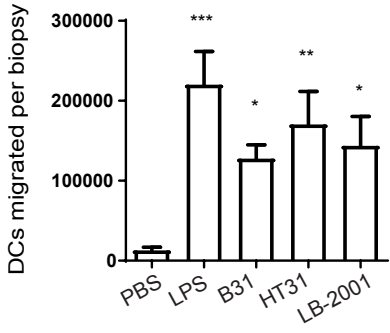
463


A
DCs alone
DCs + Borrelia
CFSE+
Count
CFSE
HT31
LB-2001
B31
360 min
60 min
10 min
0 min

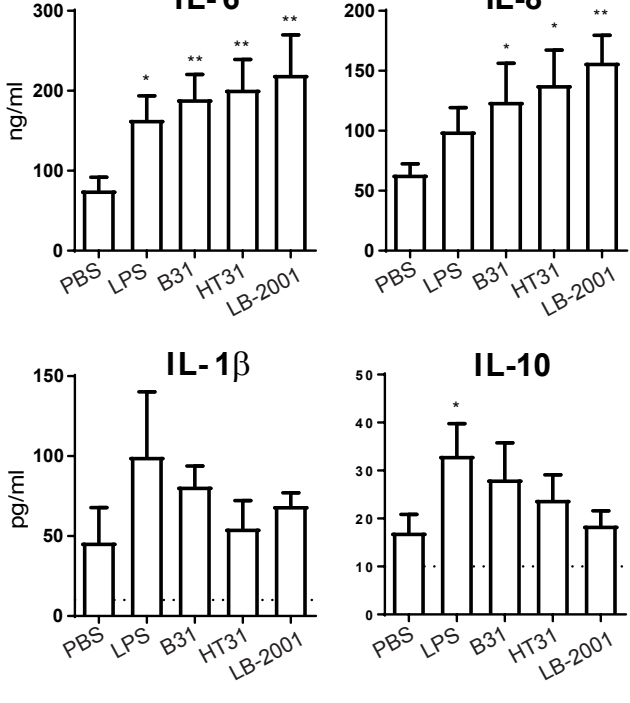

B
MOI 5
MOI 50
Phagocytosis index
Time (min)
HT31
LB-2001
B31

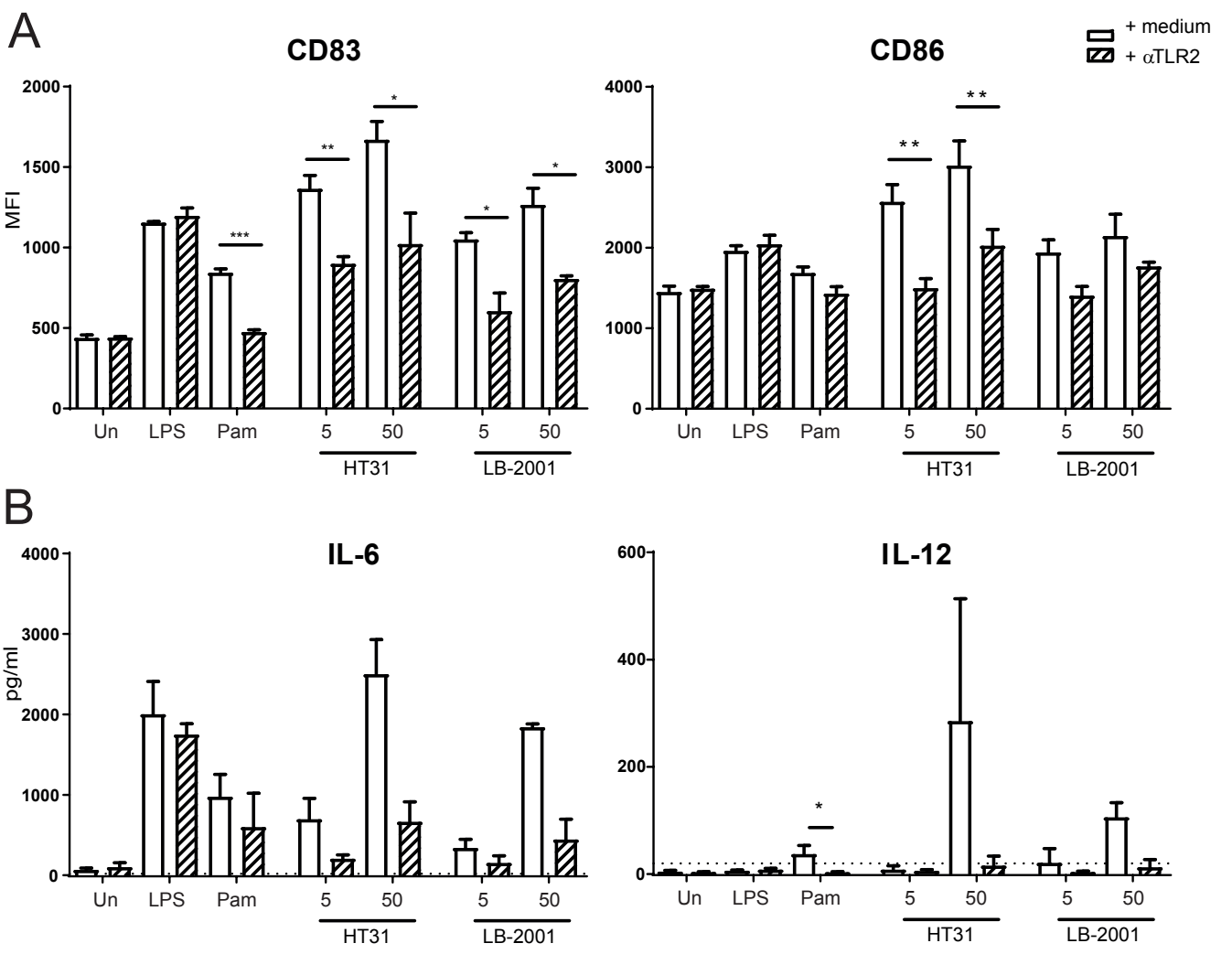

A
Unstimulated
MOI 5
MOI 50
B31
HT31
LB-2001
CD83
CD86
HLA-DR
B
CD83
CD86
HLA-DR
MFI (fold difference)
Un
LPS
5
50
B31
HT31
LB-2001
C
IL-8
IL-6
TNFα
IL-12
IL-1β
ng/ml

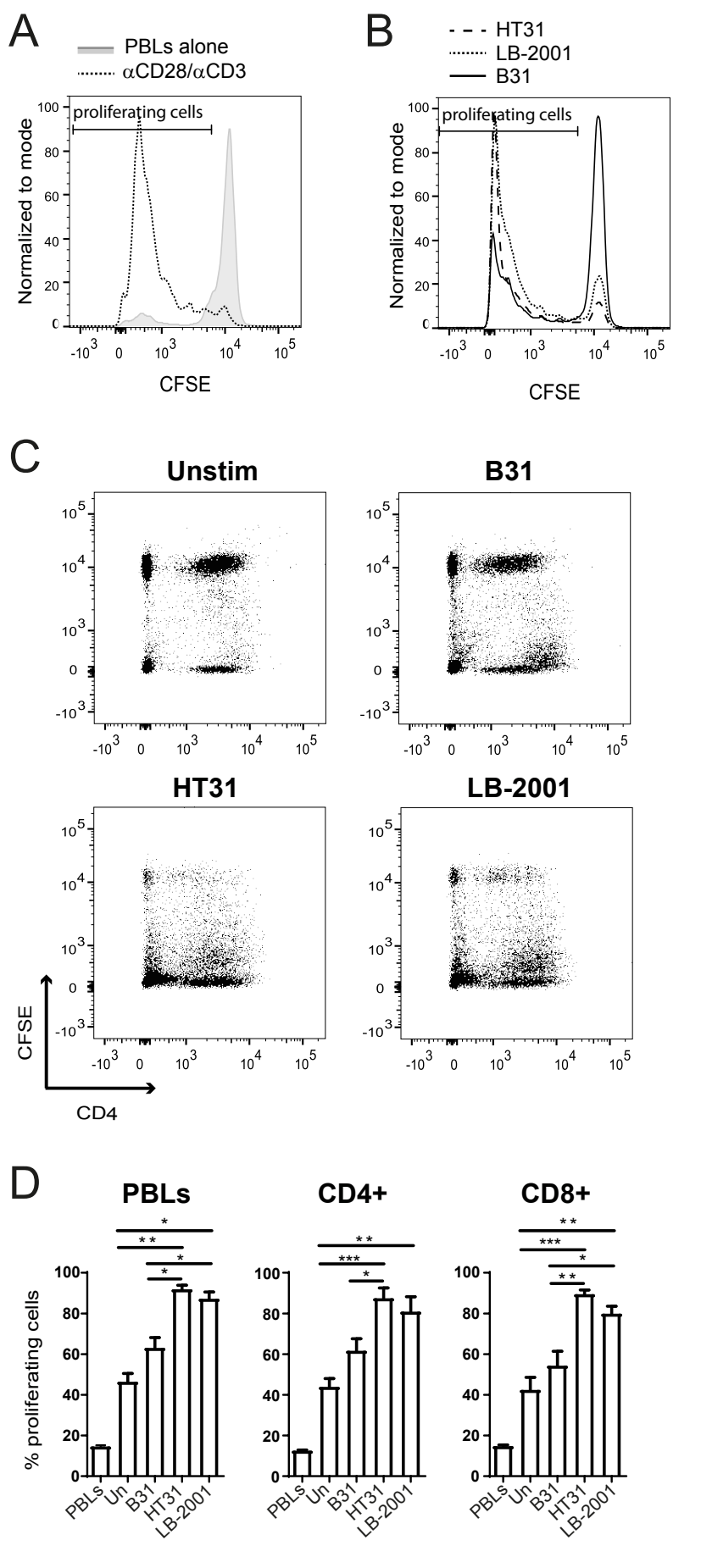
A

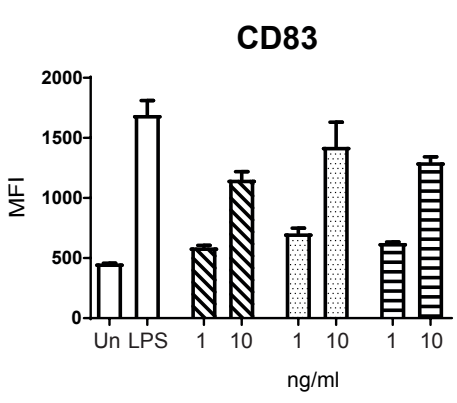

DCs migrated per biopsy
300000
200000
100000
0
***
*
**
*
PBS
LPS
B31
HT31
LB-2001

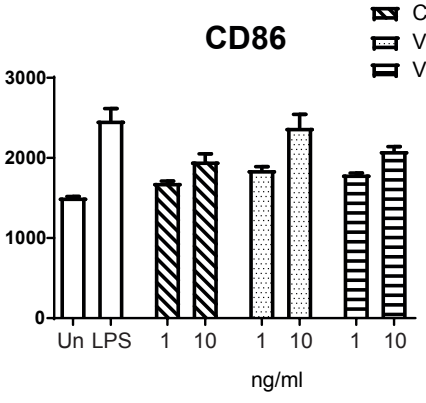
B


IL-6
ng/ml
300
200
100
0
*
**
**
**
PBS
LPS
B31
HT31
LB-2001
IL-8
200
150
100
50
0
*
*
**
PBS
LPS
B31
HT31
LB-2001
IL-1β
pg/ml
150
100
50
0
PBS
LPS
B31
HT31
LB-2001
IL-10
50
40
30
20
10
0
*
PBS
LPS
B31
HT31
LB-2001


A
CD83
CD86
+ medium
+ αTLR2
MFI
2000
1500
1000
500
0
4000
3000
2000
1000
0
Un
LPS
Pam
5
50
HT31
LB-2001
B
IL-6
IL-12
pg/ml
4000
3000
2000
1000
0
600
400
200
0


A
PBLs alone
αCD28/αCD3
proliferating cells
Normalized to mode
CFSE
B
HT31
LB-2001
B31
proliferating cells
Normalized to mode
CFSE
C
Unstim
B31
HT31
LB-2001
CFSE
CD4
D
PBLs
CD4+
CD8+
% proliferating cells
PBLs
Un
B31
HT31
LB-2001





Control
Vsp1-expressing
Vlp2c-expressing