The characteristics of *Borrelia hermsii* infection in human hematopoeitic stem cell-engrafted mice mirror those of human relapsing fever

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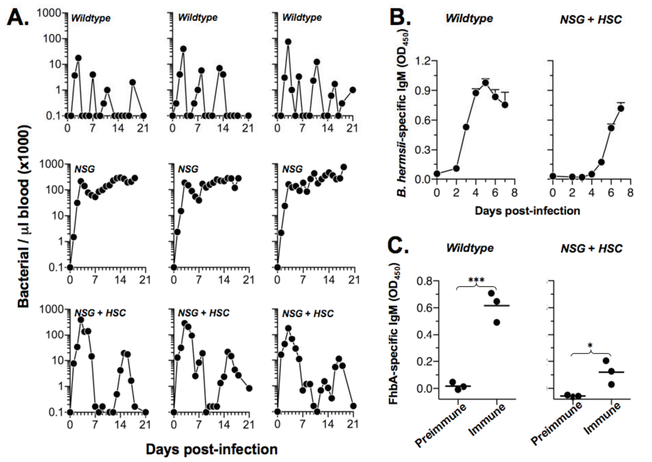
Abstract

Rodents are natural reservoirs for a variety of species of *Borrelia* that cause relapsing fever in humans. The murine model of this disease recapitulates many of the clinical manifestations of the human disease and has revealed that T cell-independent antibody responses are required to resolve the bacteremic episodes. However, it is not clear whether such protective humoral responses are mounted in humans. We examined *B. hermsii* infection in human hematopoeitic stem cell engrafted non-obese diabetic/SCID/IL-2Rγ^{null} (NSG) mice: "human immune system" mice (HISMice). Infection of NSG mice, that are severely deficient in lymphoid and myeloid compartments, with a clinical isolate of *B. hermsii*, resulted in persistent bacteremia. In contrast, this infection in HISMice resulted in recurrent episodes of bacteremia, the hallmark of relapsing fever. The resolution of the primary episode of bacteremia was concurrent with the generation of *B. hermsii*-specific human IgM. Remarkably, HISMice generated antibody responses to the *B. hermsii* outer-membrane protein FhbA. Sera from humans infected by *B. hermsii* have a similar reactivity and studies in mice have shown that this response is generated by the B1b cell subset. HISMice contain several B cell subsets including those with the phenotype (CD20+CD27+CD43+CD70-) consistent with human equivalent of mouse B1 cells. Reduction of B cells by administration of anti-human CD20 antibody resulted in diminished anti-B. hermsii responses and persistent bacteremia in HISMice. These data indicate that analysis of *B. hermsii* infection in HISMice could serve as a novel model to study the cellular and molecular mechanisms involved controlling the human relapsing fever.

Results

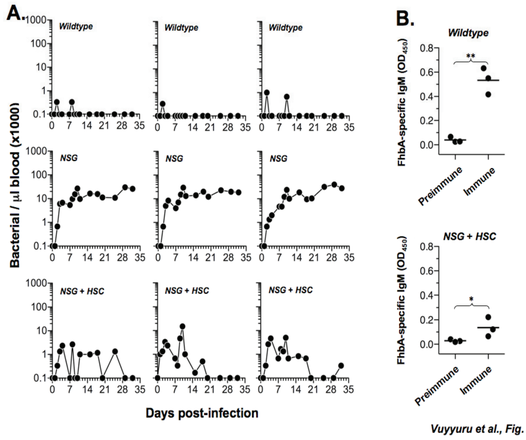
Recurrent episodes of *B. hermsii* bacteremia and generation of anti-*B. hermsii* IgM responses in HISMice.



Vuyyuru et al., Fig. 1

(A) Wildtype (C57BL/6), NOD.Cg-Prkdc^{scid}/IL2rg^{tm1Wjl}/SzJ (NSG) or human hematopoeitic stem cell-engrafted NSG (NSG+HSC) mice were infected intravenously with 5x10^4 *B. hermsii* strain DAH-p1, and bacteremia was monitored by dark-field microscopy. Each plot represents data from an individual mouse. For brevity, three representative mice from each group are shown. (B) *B. hermsii*-specific IgM generated during intravenous infection of wildtype (C57BL6) or hu-mice (NSG+HSC) was measured by ELISA. Mean ± SD values are shown. (C) FhbA-specific IgM in preimmune (Day 0 post-infection) or immune (2-3 weeks post-infection) mice following intravenous infection was measured by ELISA. Data points represent individual animals, and horizontal bars represent means of each group. Statistical significance of genotype was measured by students t-Test. *** and * denotes p<0.001 and p<0.05, respectively.y flow cytometry

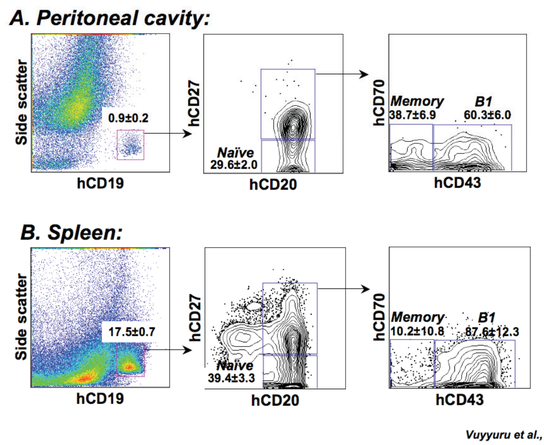
HISMice efficiently control a moderately virulent strain of *B. hermsii.*



Vuyyuru et al., Fig. 2

(A.) Wildtype (C57BL/6), NOD.Cg-Prkdc^{scid}/IL2rg^{tm1Wjl}/SzJ(NSG) or human hematopoeitic stem cell-engrafted NSG (NSG+HSC) mice were infected intraperitoneally with 5x10^4 B. hermsii strain DAH-p19, and bacteremia was monitored by dark-field microscopy. Each plot represents data from an individual mouse. For brevity, three representative mice from each group are shown. (B) FhbA-specific IgM in preimmune (Day 0 post-infection) or immune (2-3 weeks post-infection) mice following i.p. infection was measured by ELISA. Data points represent individual animals, and horizontal bars represent means of each group. Statistical significance of genotype was measured by students t-Test. *** and * denotes p<0.001 and p<0.05, respectively.

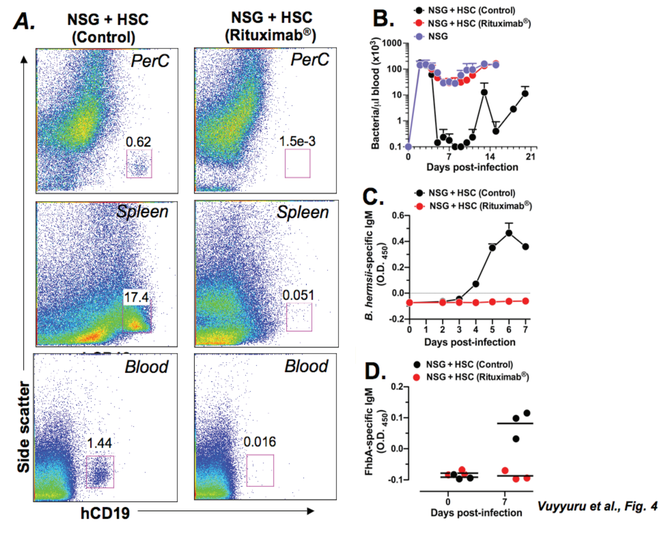
Analysis of B cells in the spleen and peritoneal cavity of HISMice.



Vuyyuru et al., Fig. 3

(A.) Peritoneal cavity cells (B.) spleen cells were stained with antibodies specific for human CD19, CD20, CD27, CD43, CD70 and CD69 and analyzed by flow cytometry. All B cells were first identified by CD19-positivity and were further resolved (indicated by arrows) as naïve (CD20+CD27-), memory B cells (CD20+CD27+ CD43- CD70-) and B1 cells (CD20+CD27+CD43+CD70-). As described previously (Griffin et al., 2011) majority of these B1 cells expressed CD5 (data not shown). The frequency values of the indicated B cell populations were shown within the plots. The data were generated by analyzing a minimum of 20,000 cells and are representative of 3-5 mice. Five percent contour plots are shown.

Depletion of B cells in HISMice results in diminished anti-*B. hermsii* responses



Vuyyuru et al., Fig. 4

(A.) HuMice treated (n=3) with or without (n=3) anti-CD20 (Rituximab®) and B cell populations in blood, peritoneal cavity and spleen were determined using and human CD19 antibodies. Representive FACS plot from one mouse was shown. Control or Rituximab®-treated mice were infected intravenously with *B. hermsii* DAH-p1 and (B) bacteremia, (C) anti-*B. hermsii*-specific IgM, and (D) anti-FhBA-specific IgM was determined.

Conclusions

- We found efficient generation of human B1 cell subsets from human HSCs in an in vivo model i.e. the HISMice system.
- *B. hermsii* infection of huMice recapitulates key bacteriological and immunological characteristics of the human disease.
- Our data indicate that analysis of *B. hermsii* infection in huMice could reveal the human functional equivalents of murine B1 subsets.

Acknowledgements

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