Brief communication

Splenic autotransplantation restores IL-17 production and antibody response to *Streptococcus pneumoniae* in splenectomized mice

B.F. Fernandes a, A.B. Rezende a, C.C.S. Alves a, F.M. Teixeira a, R.E. Farias b, A.P. Ferreira a, H.C. Teixeira a,⁎

a Department of Parasitology, Microbiology and Immunology, Biological Sciences Institute, Federal University of Juiz de Fora, 36036-900 Juiz de Fora, Minas Gerais, Brazil
b Department of Morphology, Biological Sciences Institute, Federal University of Juiz de Fora, 36036-900 Juiz de Fora, Minas Gerais, Brazil

**ABSTRACT**

The high incidence of overwhelming postsplenectomy infection caused by *Streptococcus pneumoniae* can be reduced by splenic autotransplantation. In this study the effect of splenectomy and splenic autotransplantation on the immune response to *S. pneumoniae* infection was investigated. Balb/c mice were divided into three groups: splenectomized (SP), splenectomized and autotransplanted (AT), and sham operated control (CT). Five days post-infection the serum antibody levels were measured and the number of *S. pneumoniae* CFU, neutrophil accumulation and IL-17 production in the liver and lungs were investigated. SP mice showed greater number of bacteria in both organs and lower serum levels of *S. pneumoniae*-specific IgM, IgG1 and IgG2a antibodies. IL-17 production and neutrophil recruitment to the liver and lungs were lower in SP mice, in comparison with both the CT and the AT groups. Levels of *S. pneumoniae*-specific IgM, CFU counts, neutrophil accumulation and IL-17 production did not differ significantly between the CT and AT groups. These results suggest that splenic autotransplantation restores the capacity of splenectomized mice to fight *S. pneumoniae* infection.

© 2009 Elsevier B.V. All rights reserved.

Encapsulated microorganisms are frequently involved in sepsis, which occurs more commonly in patients who have undergone total splenectomy. *Streptococcus pneumoniae*, a Gram positive bacterium, is the etiological agent in approximately 80% of cases of overwhelming post-splenectomy infection (OPSI) [1]. The high incidence of OPSI observed in splenectomized individuals increased the use of conservative methods in splenic surgery [2]. One of these methods is splenic autotransplantation, a simple technical procedure aimed to compensate for the absence of an intact spleen when its total removal is inevitable [3–6]. In splenectomized patients the production of IgM and opsonins is defective but necessary for efficient phagocytosis and elimination of encapsulated microorganisms [7,8]. The virulence of *S. pneumoniae* is in part related to its capsular polysaccharides, which impair phagocytosis mediated by antibodies and proteins of the complement system [9,10]. Recent studies demonstrate a possible involvement of IL-17 in the pathogenesis of *S. pneumoniae*. IL-17 plays a critical role in the recruitment of neutrophils to the site of infection, as well as in the formation of abscesses and in the induction of antimicrobial peptides [11,12]. This study evaluates the effect of splenectomy on the resistance of Balb/c mice to infection with *S. pneumoniae* and investigates whether splenic autotransplantation of previously splenectomized mice modulates IL-17 production, neutrophil accumulation and antibody responses after *S. pneumoniae* infection.

Female Balb/c mice from 8 to 10 weeks old were divided into three groups of 11 mice each as follows: (i) the control group (CT), mice were subjected to midline laparotomy with subsequent laparorraphy; (ii) the splenectomized group (SP), mice were subjected to splenectomy; and (iii) the autotransplanted group (AT), mice were subjected to splenectomy and splenic autotransplantation into the retroperitoneum. The mice received 0.12 ml of an anesthetic solution (0.9% NaCl, 2% xylazine, 5% ketamine) intraperitoneally before the execution of a midline laparotomy, splenectomy and binding of the vascular pedicle and short vessels using 5.0 catgut (Shalon, Goiânia, Brazil). The excised spleens were cut into six slices approximately 2.5 mm thick and kept in PBS at room temperature. In the AT group, the retroperitoneum was opened near the left kidney, and two slices were placed in the proximity of the large abdominal blood vessels without fixation. Skin closure was performed using a 4.0 running polyglactin suture. In the CT group, midline laparotomy and spleen mobilization were performed with subsequent laparorraphy. The project was approved by the Committee for Ethics in Animal Experimentation of the Federal University of Juiz de Fora (no. 59/2007).

Thirty days after surgery, six mice of each group were infected intravenously with 10⁶*S. pneumoniae* (ATCC 6303) obtained from the National Institute of Quality Control in Health (Oswaldo Cruz foundation, Rio de Janeiro, Brazil). The mice were killed 5 days after infection. Lung and liver fragments from individual animals...
were homogenized in PBS, serially diluted 10-fold and plated on trypticase soy agar (Isofar, Duque de Caxias, Brazil) enriched with 5% of defibrinated sheep blood. This medium was dispensed into six-well plates (Corning Inc., Corning, NY, USA), and incubated at 37 °C. CFU numbers were counted visually after 24 h of culture. Sera from CT, AT and SP mice were obtained and used for the evaluation of IgM, IgG1 and IgG2a isotype levels using ELISA as described previously [6]. For histologic evaluation, fragments of liver and lungs were fixed in 10% formaldehyde, processed for 14 h and then embedded in paraffin. Histologic sections about 4 μm thick were obtained from the paraffin blocks and dyed with hematoxylin-eosin (H&E). All the material was evaluated by the same observer who considered the degree of cellular infiltration in 40 fields, which was classified as low (+), moderate (+++) or intense (++++) (Fig. 1). The SP group showed a larger number of S. pneumoniae CFU in lungs (8.6 LN CFU) and liver (7.6 LN CFU) (P<0.05), in comparison with both the CT and the AT groups. The CT and the AT groups had similar CFU counts (Fig. 1A). This data suggests that splenectomy impairs the host immune response to S. pneumoniae infection and that the response can be restored by splenic autotransplantation. SP mice showed the lowest serum levels of IgM, IgG1, IgG2a, and reduced IL-17 production and neutrophil accumulation in the liver and lungs (Fig. 1B, C and D). Although the CT and the AT groups did not differ significantly in the levels of IgM, the levels of anti-S. pneumoniae IgG1 and IgG2a antibodies were significantly higher in the AT group (Fig. 1B). Cuts of hepatic and pulmonary tissue colored with hematoxylin-eosin confirmed that the animals of the SP group presented a discrete cellular infiltration, in relation to an intense cellular infiltration observed in the AT group (Fig. 2). The AT and the CT groups had similar histological characteristics (data not shown).

The principal results obtained in this work are: (i) SP mice had more S. pneumoniae CFU in liver and lungs in comparison to the CT and AT animals five days after infection, (ii) SP mice showed a lower anti-S. pneumoniae IgM, IgG1 and IgG2a antibody response, and (iii) the higher bacterial load observed in SP mice correlated both with a reduced production of IL-17 in the liver and lungs and with a lower accumulation of neutrophils at the site of infection.

Our results are in agreement with previous studies indicating that splenectomy predisposes the host to more severe bacterial infections [13,14]. To our knowledge this is the first study demonstrating that splenectomy increases the host’s capacity to respond to S. pneumoniae infection. Recent studies demonstrated that the absence of a spleen reduces serum levels of pathogen-specific IgM, which correlates with lower control over the growth of encapsulated and non-encapsulated bacteria [14,15]. In agreement with that, this work shows that S. pneumoniae-specific IgM, IgG1 and IgG2a antibody production is markedly reduced in SP mice and is restored after splenic autotransplantation. Intranasal infection with S. pneumoniae may induce an immune response independent of antibodies and dependent on CD4+ T cells [12,16]. However, after intravenous infection, which may closely resemble that observed in OPSI, antibodies appear to be critical for an effective immune response to a disseminated infection with S. pneumoniae [7,8].

Several studies demonstrate the important role of neutrophils in the control of sepsis [17,18]. The main stimulus that drives neutrophil migration to the site of infection was recently connected with IL-17 production [19–21]. In this work, the IL-17 levels were the lowest in the splenectomized group, which correlated with a reduced recruitment of neutrophils to the lungs and liver of the infected animals. These results suggest that a greater capacity for neutrophil recruitment and larger IL-17 production favor elimination of more bacteria in infected CT and AT mice. To our knowledge this is the first

**Fig. 1.** Effect of splenectomy and splenic autotransplantation on immune response to Streptococcus pneumoniae infection. Groups of Balb/c mice were infected intravenously with 10^6 S. pneumoniae 30 days after surgery. At day 5 post-infection, the number of colony-forming units per lung and liver (A), serum levels of S. pneumoniae-specific IgM, IgG1 and IgG2a antibodies (B), IL-17 production (C) and the percentage of CD11b^+ CD69^+ cells (D) per lung and liver were determined. Data represent means of six mice from a representative experiment. CT = control group (white bar), SP = splenectomized group (grey bar), AT = autotransplanted group (black bar). *P<0.05 versus CT and AT groups; #P<0.05 versus CT group.
report covering IL-17 production, splenectomy and resistance to S. pneumoniae infection.

In conclusion, splenic autotransplantation seems to favor a better control of S. pneumoniae growth in liver and lungs of splenectomized BALB/c mice. This appears to be associated with increased IL-17 production at the site of infection and high serum levels of specific antibodies, which together may facilitate phagocytosis and elimination of bacteria by recruited inflammatory cells.

References


Fig. 2. Photomicrograph showing inflammatory infiltrations stained with hematoxylin-eosin in liver (A and B) and lung (C and D) of S. pneumoniae infected splenectomized (A and C) and autotransplanted (B and D) mice. (A, B×400; C, D×200).