Role of Spleen in Morbidity and Mortality of *Plasmodium berghei* Infection in Mice

WIJNAND M. C. ELING

Department of Cytology and Histology, Faculty of Medicine, Catholic University of Nijmegen, 6500 HB Nijmegen, The Netherlands

Splenectomy has a strain-specific effect on *Plasmodium berghei* infection in mice. Mean survival time either was unchanged or increased to three times the value observed in intact controls. A delay of early mortality, which was otherwise observed in the second week of infection, was a general feature of susceptible strains. Delayed mortality was also observed when splenectomy was performed shortly before expected mortality. Ineffectiveness of splenectomy as to increased survival time was independent of the infective dose. The morbidity of the infection was reduced or delayed. Liver pathology, as assayed by changes in serum glutamate oxaloacetate transaminase activity, was always reduced after splenectomy. Strains exhibiting increased survival time after splenectomy also showed reduced peak parasitemia and delayed thymus involution, enhanced reduction of hematocrit, and a more pronounced increase in liver weight during infection compared to intact controls and strains in which splenectomy did not prolong survival times. The effect of splenectomy on morbidity and mortality of a *P. berghei* infection was compared to its strain-specific effect on antibody production to heterologous erythrocytes. The possibility of a spleen-dependent (immuno-) pathological response induced by the parasite during primary infection contributing to death of the host is discussed.

The spleen is considered an important organ and site for immunological response against antigens and pathogens circulating in the blood (9). Splenectomy usually impairs innate immunity as well as induction and expression of acquired immunity against several types of malaria parasites in mammals and birds (19). Hosts with innate resistance become susceptible after splenectomy (19), mild, transient infections become virulent and fatal in splenectomized hosts (16, 17, 21), and immune hosts may lose protection after splenectomy (1, 2, 20) although the situation may vary considerably in different experimental models (7). These observations have focused attention on the immunological role of the spleen in defense against malaria. Less attention has been paid to a decreased morbidity of infections in splenectomized hosts, e.g., increased survival time and delayed anemia (6, 16, 17).

This report describes a strain-specific effect of splenectomy on a virulent and lethal primary infection of *Plasmodium berghei* in mice. The results indicate that in this model splenectomy frequently decreased and delayed morbidity of the infection, suggesting an active role of the spleen in the generation of a (immuno)pathological reaction during a primary infection in the intact animal.

MATERIALS AND METHODS

Parasite strain. The parasite, *P. berghei*, strain K-173, was maintained by sub-inoculation of 10⁶ parasitized erythrocytes (PE) into clean mice of the corresponding strain and sex at weekly intervals. Parasitemia was determined from thin smears made from tail blood and stained with May Grünwald-Giemsa solutions.

Mice. Mice used in the experiments were 6 to 8 weeks old and were kept in plastic cages on standard food (RMH Hope Farms) and water ad libitum. Outbred Swiss and inbred B10LP, C3H/STZ, BALB/c, and C57BL/RIJ mice were obtained from the animal facilities of the University of Nijmegen. B10LP nu/+ mice were obtained from TNO Zeist, The Netherlands.

Calculation of parameters. Groups of four, or sometimes (during infection) three, mice were used to determine pathophysiological parameters. In each group, the geometric mean value and standard deviation were calculated.

Mortality patterns and mean survival times. Mortality patterns and mean survival times of the *P. berghei* infection in mice of the different strains were determined in groups of 20 mice left undisturbed after intraperitoneal infection with 10⁵ PE. Mortality was scored per day.

Hematocrit values. Hematocrit values were determined from blood obtained by puncturing the orbital plexus of the mice under ether anesthesia.

Serological tests. Serum glutamic oxaloacetic
transaminase (SGOT) activity (aspartate aminotransferase activity) was determined in fresh serum by an ultraviolet test (biochemical tests, Boehringer Mannheim Corp.).

Splenectomy. Splenectomy was performed 2 weeks before infection, unless otherwise indicated. After a lateral incision, the splenic pedicles were ligated, the spleen was removed, the body wall was sutured, and the skin was closed with Michell clamps. The whole procedure was carried out under ether anesthesia.

Hemagglutinating antibody responsiveness. The hemagglutinating antibody responsiveness against rabbit erythrocytes was determined in splenectomized and intact control mice. Freshly drawn, leukocyte-free rabbit erythrocytes were used as antigen. Each mouse received 0.2 ml of a 10% (hematocrit) suspension of rabbit erythrocytes intraperitoneally, and serum was collected for analysis 7 days later. The amount of hemagglutinating antibody produced was determined by routine procedures (5), using 2-mercaptoethanol for differentiation of immunoglobulin M (IgM) and IgG antibodies. In each experiment, the geometric mean value of the results obtained from groups of four mice was calculated.

RESULTS

Effect of splenectomy on survival time of a primary infection. The effect of splenectomy performed before or during primary infection on the survival period of mice was determined in several mouse strains in comparison with intact controls or sham-operated mice. Since no differences were observed between intact controls and sham-operated mice, the results of these groups were pooled (Fig. 1).

The effect of splenectomy was found to be strongly strain dependent. Splenectomy had no discernible effect on the mean survival time of Swiss and C3H/StZ mice. The tendency for longer survival of C3H/StZ mice splenectomized later during infection is primarily a result of the selection of mice surviving longest during a primary infection. In the strains tested, the strongest effect of splenectomy was observed in splenectomized B10LP mice with a mean survival time three times longer. This remarkable increase was observed in B10LP mice splenectomized before, as well as during, infection. Even splenectomy at peak parasitemia (day 7; cf. reference 12) or later, when mortality was observed in control mice, had the effect of delaying mortality. Many of these mice were very ill and did not survive splenectomy. The increase in mean survival time of splenectomized, infected BALB/c, B10LP nu/+, and C57BL/Rij mice was intermediate. Results were not always significant, due to a high standard deviation, especially in intact controls (e.g., C57BL/Rij mice). Therefore, the effect of splenectomy on the mortality pattern was considered as well (Fig. 2).

The effect of splenectomy on the mortality pattern of primary infection was strongly strain dependent: either the whole pattern shifted (B10LP, B10LP nu/+, BALB/c), or the early peak of mortality normally occurring during the second week of infection shifted (B10LP nu/+, BALB/c, C57BL/Rij), or splenectomy had no significant effect (Swiss). Results with C3H/StZ mice (data not shown; cf. Fig. 1) were compara-

![Fig. 1. Survival in relation to splenectomy before or during infection. Groups of mice were splenectomized or served as controls, either 14 or 1 day(s) before infection (20 mice per group), or 4 to 15 days after infection with 10^6 PE (number of mice per group varied from 2 to 7). Mortality was scored per day, and the geometric mean value and standard deviation (only in groups of more than two mice) was calculated and shown in the figure, except that the standard deviation of the groups splenectomized 14 days before infection was omitted for better legibility.](http://iai.asm.org/)
uble to those of Swiss mice. The mortality pattern also shows that the high standard deviation of the mean survival time of, e.g., C57BL/Rij mice is due to the biphasic mortality which is a characteristic of primary \textit{P. berghei} infection in some mouse strains (3).

Survival may be related to the development of immune responses (protective or deleterious) against the parasite. Since an immune response against a spleen-processed antigen is inhibited by splenectomy but returns when increased amounts of antigen are applied (10), the possibility was investigated that increased survival in splenectomized mice was related to the infective dose. Swiss mice were used for these experiments, because splenectomy had no discernible effect on the mean survival time under standard conditions (10^5 PE). The results of these experiments are shown in Fig. 3. In the range of 10^2 to 10^7 PE injected per mouse at 14 days after splenectomy or in intact controls, a close correlation was observed between an increase in mean survival time and a decreasing infective dose, independent of previous splenectomy.

**Effect of splenectomy on morbidity of a primary infection.** The above-described experiments showed that splenectomy either resulted in a more or less distinctly delayed mortality or had no discernible effect. In a subsequent experiment, it was determined whether pathophysiological changes otherwise involved in a primary infection were also influenced by splenectomy. Since such changes are prominent on day 7 after infection with 10^5 PE, measurements were started on this day.

Swiss mice were selected to represent strains that have no discernible change in mortality after splenectomy, and B10LP mice were se-

![Fig. 2. Cumulative mortality in intact (——) and splenectomized (—-) mice infected with \textit{P. berghei}. Groups of 20 mice, either intact or splenectomized, were infected (10^6 PE) 14 days later. Mortality was scored per day and shown in the figure as cumulative mortality.](http://iai.asm.org/)

![Fig. 3. Effect of infective dose on mean survival time in intact (x) and splenectomized (o) \textit{3} Swiss mice. Groups of 10 mice were either splenectomized or served as controls, and parallel groups of splenectomized and intact mice were infected with doses ranging from 10^6 to 10^7 PE per mouse 14 days later. Two mean survival time (± standard deviation) was calculated from the mortality per day.](http://iai.asm.org/)
lected to represent strains with prolonged survival after splenectomy. Groups of 20 mice either served as controls or were splenectomized, and 14 days later all mice were infected (10⁸ PE). On days 7, 8, 9, 11, and 14 after infection, parasitemia, hematocrit, relative thymus and liver weights, and SGOT activity were determined. The results are shown in Fig. 4.

Splenectomy reduced parasitemia in B10LP mice and possibly also in Swiss mice, but in the latter this was insignificant due to the associated high standard deviations. Hematocrit was lower in splenectomized B10LP but not in splenectomized Swiss mice. Whereas thymus involution was considerably less in splenectomized B10LP mice, the difference between splenectomized and control Swiss mice was less and again, due to high standard deviations, not significant.

Splenectomy resulted in a considerable increase in liver weight of B10LP mice, and this increase was much less in controls. Although an increase in liver weight was observed in infected and splenectomized, infected Swiss mice, there was no discernible effect after splenectomy. The SGOT activity was strongly reduced after splenectomy, and despite a less prominent effect in splenectomized Swiss mice, again because of high standard deviations, peak high values, normally observed at peak parasitemia (7 days after infection), were absent.

In summary, a splenectomy-dependent suppression of SGOT activity correlated neither with increased survival nor with changes in organ weight. In B10LP mice, the increased survival after splenectomy was associated with more or less distinct changes in liver and thymus weight, hematocrit, and possibly parasitemia, whereas minimal or no changes were found in splenectomized Swiss mice.

**Effect of splenectomy on immunological responsiveness.** Mortality and morbidity of a primary infection may be related to development of immune responses (protective or deleterious) against the parasite or its product. To test this possibility, responsiveness against heterologous erythrocytes was determined in splenectomized and intact control mice of different strains. The results of these experiments are given in Table 1. Since the magnitude of the total immunoglobulin, as well as the IgG responses, was strain specific, the percent reduction of both the total immunoglobulin and the proportional IgG responses was calculated to compare the effect of splenectomy in different strains.

The responsiveness against rabbit erythrocytes was reduced after splenectomy in all strains tested. The reduction was strain specific, being strongest in c57BL/Rij mice and least in Swiss mice. The effect of splenectomy on production of IgG-type agglutinating antibody was even more remarkable (Table 1).

**Table 1. Reduction in the amount of anti-rabbit erythrocyte antibody in splenectomized versus intact mice**

<table>
<thead>
<tr>
<th>Mice</th>
<th>Reduction (%) of immunoglobulin response</th>
<th>Reduction (%) of proportional IgG response</th>
</tr>
</thead>
<tbody>
<tr>
<td>c Swiss</td>
<td>38 (3)</td>
<td>0</td>
</tr>
<tr>
<td>o Swiss</td>
<td>37 (1)</td>
<td></td>
</tr>
<tr>
<td>c C3H/Stz</td>
<td>56 (3)</td>
<td>0</td>
</tr>
<tr>
<td>c BALB/c</td>
<td>59 (2)</td>
<td>44</td>
</tr>
<tr>
<td>o B10LP</td>
<td>64 (5)</td>
<td>36</td>
</tr>
<tr>
<td>o c57BL/Rij</td>
<td>54 (2)</td>
<td></td>
</tr>
<tr>
<td>c C57BL/Rij</td>
<td>76 (3)</td>
<td>100</td>
</tr>
</tbody>
</table>

*a* The number in parentheses refers to the number of independent determinations.

*b* Reduction (%) of the ratio IgG/IgM + IgG after splenectomy.
of IgG antibody was blocked in splenectomized C57BL/Rij mice, whereas in Swiss and C3H/StZ mice the fraction of IgG out of the total amount of antibody remained unaffected, reduction of the amount of IgG being proportional to reduction of total amount of antibody. In B10LP and BALB/c mice, the reduction in the proportion IgG response after splenectomy was intermediate.

In additional experiments (not shown), the effect of a higher dose of antigen on the amount of antibody produced in splenectomized mice was investigated. The results of two independent experiments with female Swiss and C57BL/Rij mice showed that the total amount of antibody, as well as the IgG fraction, remained the same when three times the amount of antigen was injected.

In other experiments (not shown), longitudinal observations showed that splenectomy reduced rather than delayed responsiveness.

**DISCUSSION**

The spleen plays an important role in malaria. It contributes to innate resistance and is important for the induction and expression of acquired immunity (for a review, see reference 19). Usually splenectomy has an adverse effect on the host's defense against the parasite, and infections become more severe in splenectomized humans (3) and in animals, such as monkeys, birds, and rodents (6, 14–17, 19). Less attention has been paid to decreased morbidity of a primary infection in splenectomized mice, although several indications are found in the literature. Restricting ourselves to the virulent P. berghei mouse model, we find that Kretschmar and Jerusalem observed a slightly increased mean survival time in splenectomized mice and compared it to introc mice (6), but this was not observed by Topley et al. (17) or Singer (14). Our results (Fig. 1 and 2) show that increased survival after splenectomy is dependent on the mouse strain and may range from ineffective to a three-times prolonged mean survival. Reduction of the mean survival time after splenectomy was not observed in this virulent parasite-mouse model, suggesting that strain-specific differences in survival periods of intact animals apparently are not related to differences in immunological potential (better responders survive longer). Increased survival periods after splenectomy could indicate that a spleen-dependent and parasite-induced immunopathological response is involved in mortality in infected, intact animals.

Involvement of the spleen in a deleterious reaction in infected intact B10LP mice is strongly suggested by the observed increased survival time, when splenectomy is performed shortly before expected mortality (Fig. 1).

In general, at least a delay of the early phase of mortality was observed in splenectomized animals of appropriate parasite-mouse combinations (Fig. 2). Early mortality in the beginning of the second week of infection may coincide with the period needed for an immunopathological response to become effective. Such a hypothesis may be further supported by decreased morbidity observed in splenectomized infected mice (Fig. 4), being at least partly related to increased survival. On the other hand, an impressive increase in SGOT activity, which is considered to be a marker for liver injury (3, 8, 11), is reduced after splenectomy in all strains and not correlated with increased survival. Either several, mutually independent responses or the involvement of non-immunological, e.g., rheological mechanisms (19) or differences in erythropoietic capacity (cf. 4), may have to be considered.

Thymic involution is a prominent feature of P. berghei, as well as many other malaria infections (18), and may be part of deleterious changes in the immune apparatus during a primary infection that give rise to immunodepression (12, 18). Thymic involution was reduced and delayed in splenectomized mice and correlated with increased survival (Fig. 4). This again suggests that in the intact infected animal a spleen-dependent (immuno)pathological reaction is involved in thymic involution.

A lower peak parasitemia (7 days after infection with 10⁵ PE) in splenectomized animals was observed by Kretschmar and Jerusalem (6) but not by Singer (14), which, in the light of results described in Fig. 4, may depend on the strain of mice used. Since the mouse spleen has an important erythropoietic function, splenectomy may inhibit proliferation of parasitic host cells, thereby reducing parasitemia. Reduced replacement of phagocytosed erythrocytes may account for decreased hematocrit values in splenectomized infected mice (Fig. 4). Likewise, the cause of the impressive increase in liver weight in splenectomized versus control B10LP mice (Fig. 4) and its possible contribution to decreased liver pathology, as determined by decreased SGOT activity (Fig. 4), need further attention.

The role of the spleen in response to malarial antigens could be compared to the response to heterologous erythrocytes (10, 19). Rowley (10) observed a decreased responsiveness in splenectomized rats that could be compensated for by an increased antigen dose. A linear correlation between the logarithm of the infective dose and the mean survival time in Swiss mice was not...
changed by splenectomy (Fig. 3).

Antibody production against heterologous erythrocytes was reduced in splenectomized, uninfected mice of all strains tested (Table 1), but the magnitude of the reduction was not correlated to the magnitude of the increase in mean survival time in infected mice after splenectomy, i.e., C3H/StZ mice exhibited a severe decrease in antibody production but no increase in survival time after splenectomy. Splenectomy had a remarkable effect on the IgG response against heterologous erythrocytes. No reduction was observed in the proportional IgG response of Swiss and C3H/StZ mice, and this coincides with ineffectiveness of splenectomy with regard to prolongation of survival, whereas in all other cases a reduction of the proportional IgG response coincided with some increase in mean survival time.

It should be noted, however, that the greatest prolongation of mean survival time observed in splenectomized B10LP mice did not correlate with the strongest reduction of the IgG response. Changes in responsiveness against heterologous erythrocytes due to splenectomy may indicate to what extent other organs are involved in antibody production or can compensate for removal of the spleen. In this light, IgG production in C57BL/10j mice, unlike other strains tested, entirely depends on the presence of the spleen, whereas the increase in mean survival time after splenectomy is only intermediate (Fig. 1 and 2). This might suggest that if a spleen-dependent, immunopathological response is involved in mortality and morbidity of a P. berghei infection in mice, and such a response is antibody mediated, we may have to look for IgM antibody.

In summary, the results show that splenectomy before or during a virulent and fatal infection of P. berghei in mice is either ineffective or results in a more or less prolonged survival time. This is in contrast to results in models with mild and transient infections or with innate resistance, in which splenectomy results in increased susceptibility, greater morbidity, and frequently fatal infection. In our model, with virulent infections splenectomy also reduced and delayed pathophysiological changes after infection, and usually did so more strongly when mortality was more strongly delayed after splenectomy, except for changes in SGOT activity, which was suppressed in all cases.

Decreased morbidity of the infection and decreased antibody production after splenectomy may suggest involvement of a spleen-dependent (immuno)pathological response in the morbidity of the infection in intact animals during primary infection. Strain-specific effects may either depend on involvement of other lymphoid organs (lymph nodes) or on the possibility of compensatory reactions in other lymphoid organs in splenectomized animals.

Since the spleen is important for the induction of antimalarial immunity (for a review, see reference 19), it is not surprising that splenectomized mice die from infection and that, with regard to pathological changes like anemia, thymus involution, and mortality, only temporary effects are observed after splenectomy. Moreover, immunosuppression observed in association with a P. berghei infection (12) may prevent compensatory reactions with regard to induction of immunity in, e.g., lymph nodes.

ACKNOWLEDGMENTS

I thank J. Koekkoek-Smeenkens, C. Hermens, J. Reitsma, G. Poelen, M. Faassen, and J. van de Weeren for skilled technical and biotechnical assistance, and M. L. Weiss for reading the manuscript.

This work received financial support from the World Health Organization, Geneva, Switzerland, and the Dutch Government through the International Cooperative Research Project on Malaria Immunity and Immunopathology.

LITERATURE CITED


