Z. Parasitenk. 54, 29–45 (1977)

Zeitschrift für
Parasitenkunde
Parasitologic Research

The Course of a *Plasmodium berghei* Infection in Six Different Mouse Strains*

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Summary. Experiments were carried out to study common and strain specific features of malaria (*P. berghei*) in mice. In the various mouse strains infection with 10^5 P.E. results in four different mortality patterns, with frequent secondary infections among all long surviving mice. A prepatent period of four days, proliferation in oxyphilic erythrocytes, a first peak of parasitaemia on day 7/8 and crisis occur in all strains. The magnitude of peak parasitaemia, however, and the ability to develop polychromatocytosis which coincides with survival beyond crisis are strain specific. Transient variations in number of erythrocytes and s.g.o.t. activity shortly after infection are the earliest noticeable changes, but do not predict later ones. The inception of definitive anaemia is on day 6 in all strains, and thus just before the first peak of parasitaemia but independent of its magnitude. Anaemia further develops at a strain specific rate. In general, parasite proliferation, peak infection, crisis, and polychromatocytosis are followed by changes in s.g.o.t. activity chronologically but not quantitatively. Extensive thymic involution occurs in all strains, but develops in a strain specific pattern. Initial changes in spleen weight can reflect R.E.S. activity and an ongoing immunological process which collapses, however, around peak infection. Subsequent increases in spleen weight may in particular depend on proliferation of erythropoietic tissue, especially in instances of distinct polychromatocytosis. There is no quantitative correlation between changes in liver weight and s.g.o.t. activity, but focal liver cell necrosis may account for high s.g.o.t. activity.

Index descriptors: rodent-malaria, *Plasmodium berghei*, mice, pathophysiology, parasitaemia, anaemia, polychromatocytosis, s.g.o.t. activity, body weight, thymus involution, thymus, liver, spleen, mortality.

Introduction

The features of malaria vary considerably with the parasite–host combination. Within a given model, e.g. *Plasmodium berghei* in rodents, and even limited to a

* These investigations received financial support from the World Health Organization, Geneva, Switzerland, and the Dutch Government through the International Cooperative Research Project for Malarial Immunity and Immunopathology.

given species, e.g. mice, great variations in the course of parasitaemia, morbidity, and mortality (see also Greenberg and Kendrick, 1957, 1958) ranging from lethal infections in many mouse strains to spontaneous recovery among N.M.R.I. mice (Kretschmar, 1962) are amply documented.

Although numerous studies dealt with histological, physiological and immunological changes in malaria (for reviews see Maegraith, 1973, 1974; Voller, 1974; W.H.O. techn. Rep. Ser. No. 579, 1975) little is known about both the aetiology of the changes and the possible interrelationships contributing to a fatal outcome. A better understanding of the correlation of processes and information on their variability in allogeneic strains would enable us to select a suitable model for an efficient analysis of a given phenomenon. It is obvious that, unlike in the randomly breeding human species, the animal model permits an analysis of a defined and reproducible course of events, especially in the case of clinical and fatal malaria. Furthermore, in the mouse model a suitable number of comparable individuals can be followed, several parasite–mouse strain combinations compared, and the specific course of the infection and mortality pattern analysed (Sengers et al., 1971a, b; Eling and Jerusalem, 1977a). Strain specificity of syndromes may either depend on genetically determined physiological factors, e.g. on the relation between the A.T.P. concentration in erythrocytes and the rate of parasite proliferation (Brewer and Powell, 1965), on the possible influence of secundarily produced kinins, peptides, histamin or adenosin (Maegraith and Onabanjo, 1969; Onabanjo and Maegraith 1970a, b; Maegraith and Fletcher, 1972), or may be determined by the host's immune responses, restricting parasite multiplication or producing auto-antibody and immunological injury (Voller, 1974). Such strain specific factors which may be reflected by changes in the haemogram, organ weight or structural and functional lesions of organ systems can be compared with those in other animals and in human malaria (Sadun et al., 1966).

Since little is documented on strain specific features of malaria (Greenberg and Kendrick, 1957, 1958) experiments were carried out to study variations in the number of (infected) erythrocytes, changes in weight of body, thymus, spleen, and liver, and serum components, like serum glutamic oxalacetic transaminase (s.g.o.t.) activity (Sadun et al., 1965, 1966), that might possibly reflect hepatic injury in several mouse strains during the whole course of the disease. An analysis of the results in the various parasite–host combinations was undertaken to show common features and strain specific courses as a correlate of genetic differences, and the possible interrelationship of the various changes.

Materials and Methods

Plasmodium berghei, strain K 173 was used in all experiments and maintained by weekly transfer (i.p.) of 10^5 parasitized erythrocytes (P.E.). This *P. berghei* infection is always lethal in mice. Female Swiss mice, bred in closed colonies, and inbred ♀ germfree Swiss, ♀ C3H/StZ and ♀ Balb/c mice were obtained from the animal facilities of the University of Nijmegen, as were inbred ♂ B10LP, and ♂ B10LP nu/nu (7th–8th backcross generation) from T.N.O., Zeist, The Netherlands. The mice were 6–8 weeks old when taken into the experiment, except for the groups of germfree Swiss and B10LP nu/nu mice which were inhomogeneous with respect to age and body weight, because they were available only in limited numbers, and animals obtained at regular intervals had to be stored in order to obtain a sufficiently large group.

All animals were kept in plastic cages on standard food (Hope Farms), and water ad libitum, under S.P.F., and germfree Swiss mice under germfree conditions. All experimental mice were inoculated intraperitoneally with 10^5 P.E. from donor mice of the same strain and sex except for B10LP nu/nu and germfree Swiss mice which received P.E. from infected B10LP and Swiss donors respectively. The

infectious inoculum for germfree Swiss mice was prepared under sterile conditions. Mortality patterns and mean survival times of the various *P. berghei*-mouse strain combinations, and pathophysiological parameters were determined in independent experiments. For the one series groups of 20 mice (Swiss, B10LP, C3H/StZ, Balb/c) 27 mice (B10LP nu/nu) and 18 mice (germfree Swiss) were infected with 10^5 P.E. and left undisturbed. An additional number of 80–100 mice of each strain in groups of 20 were infected (10^5 P.E.), and served for assessment of functional lesions. A varying number of these mice, depending on the strain, succumbed due to the fatal infection, and before analysis was possible. Immediately before and on varying days after inoculation tail blood was withdrawn from at least four mice of each group for a thin smear and erythrocyte counts. Subsequently, these mice were anaesthetized with ether, weighed, exsanguinated through the orbital plexus and sacrificed by cervical dislocation. Spleen, liver and thymus were prepared free of other tissue and weighed. Organ weights are expressed in percent of body weight. Parasitaemia was determined in May-Grünwald-Giemsa stained thin smears. For erythrocyte counts 2 μl of tail blood were diluted 50,000 times with Isoton® and erythrocytes counted with an electronic particle counter (Coulter Counter, type B). Serum glutamic oxalacetic transaminase (s.g.o.t.) activity was measured in 0.1 ml of fresh serum by means of the U.V. test (Biochemica Tests, Boehringer) determined at 340 nm in a Zeiss PMQ4 spectrophotometer, and haemoglobulin concentrations by the colourimetric cyanid method at 540 nm with known haemoglobulincyanid concentrations (Rijks Instituut Volksgezondheid, Bilthoven, The Netherlands) as standards. Of each group the geometric mean value and standard deviation were calculated. The whole set of experiments was performed twice, and since comparable results were obtained, only the data of one set are presented.

Results

Mortality and Mean Survival Times

The mortality patterns and mean survival times differ largely in the various parasite–mouse strain combinations (Fig. 1, Table 1). Swiss mice exhibit a typical bimodal pattern, with a first peak of mortality at the beginning of the second week and a

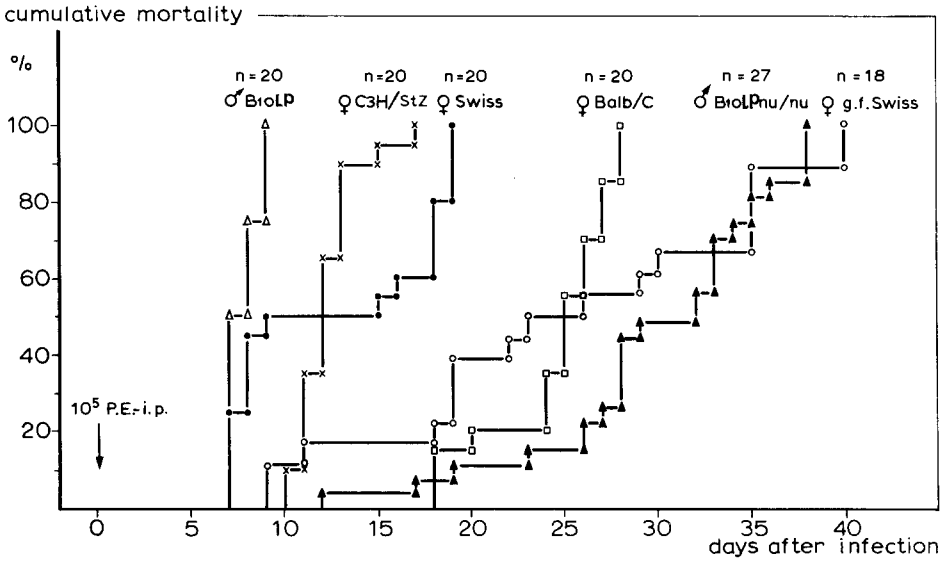

Fig. 1. Cumulative percent mortality in 6 different mouse strains after intraperitoneal inoculation with 10^5 P.E. The number of mice (= *n*) per group is indicated in the figure

second wave of mortality during the third week after infection. The first peak coincides with oxyphilic peak infection and crisis (see there).

Compared to Swiss mice three additional mortality patterns can be observed in the other strains: (a) B10LP mice all die at the beginning of the second week after infection, that is during the period when Swiss mice exhibit the first peak of mortality; (b) the mortality pattern of C3H/StZ like that of B10LP mice is monophasic but deaths occur almost entirely at a time between the first and second mortality peak of the Swiss mice; (c) in Balb/c, germfree Swiss and B10LP nu/nu mice the great majority of mice die during or after the period of the second peak of mortality in Swiss mice. In the compartively long surviving animals cumulative mortality increases gradually over a long period; the mice are in poor condition already from the second week on, and secondary infections, e.g. diarrhoea, infections of the eyes and nose are frequent.

Parasitaemia

After inoculation of 10^5 P.E. the prepatent period lasts approximately four days in all strains. The subsequent asynchronous proliferation of parasites in oxyphilic erythrocytes is also common to all strains and leads to an oxyphilic peak parasitaemia either on day 7 (Balb/c; Swiss and germfree Swiss; B10LP nu/nu) or day 8 (C3H/StZ and B10LP) after infection.

The magnitude of peak parasitaemia is virtually strain dependent (Table 1). The ensuing reduction in the proportion of infected erythrocytes (crisis) is found in all mouse strains, and takes place while the plasmodia still preferentially parasitize oxyphilic erythrocytes.

The magnitude of peak parasitaemia is apparently not related to the ability: (a) to produce crisis per sé; (b) to establish polychromatocytosis after crisis, or (c) to survive for a longer period. On the other hand, mortality is not observed before oxyphilic peak infection. A renewed increase in the percentage of infected erythrocytes is observed only when polychromatocytosis is established, and polychromatophilic erythrocytes are predominantly and to almost 100% parasitized. Now only a small number of oxyphilic erythrocytes are infected. If this switch to infected polychromatophilic erythrocytes can be established, it is achieved approximately between day 11 and 14 after infection.

With respect to polychromatocytosis two patterns can be distinguished: (a) in several parasite–mouse strain combinations the majority of the mice develop polychromatocytosis and switch from infected oxyphilic to polychromatophilic erythrocytes after crisis. In these instances the majority of the mice survive oxyphilic infection (germfree Swiss, Balb/c, B10LP nu/nu, and part of the Swiss mice); (b) in the other groups mice die either at oxyphilic peak infection or during crisis, and do not exhibit polychromatocytosis (B10LP, C3H/StZ, and part of the Swiss mice). Reduction in the proportion of infected erythrocytes during crisis can be extensive, but does not prevent death during this stage of the disease, as could frequently be observed among C3H/StZ and B10LP mice.

Those mice, however, that survive longer, i.e. longer than 14 days, exhibit crisis, polychromatocytosis and the switch to infected polychromatophilic erythrocytes.

Erythrocyte Numbers

In C3H/StZ and Swiss mice a transient increase in the number of erythrocytes

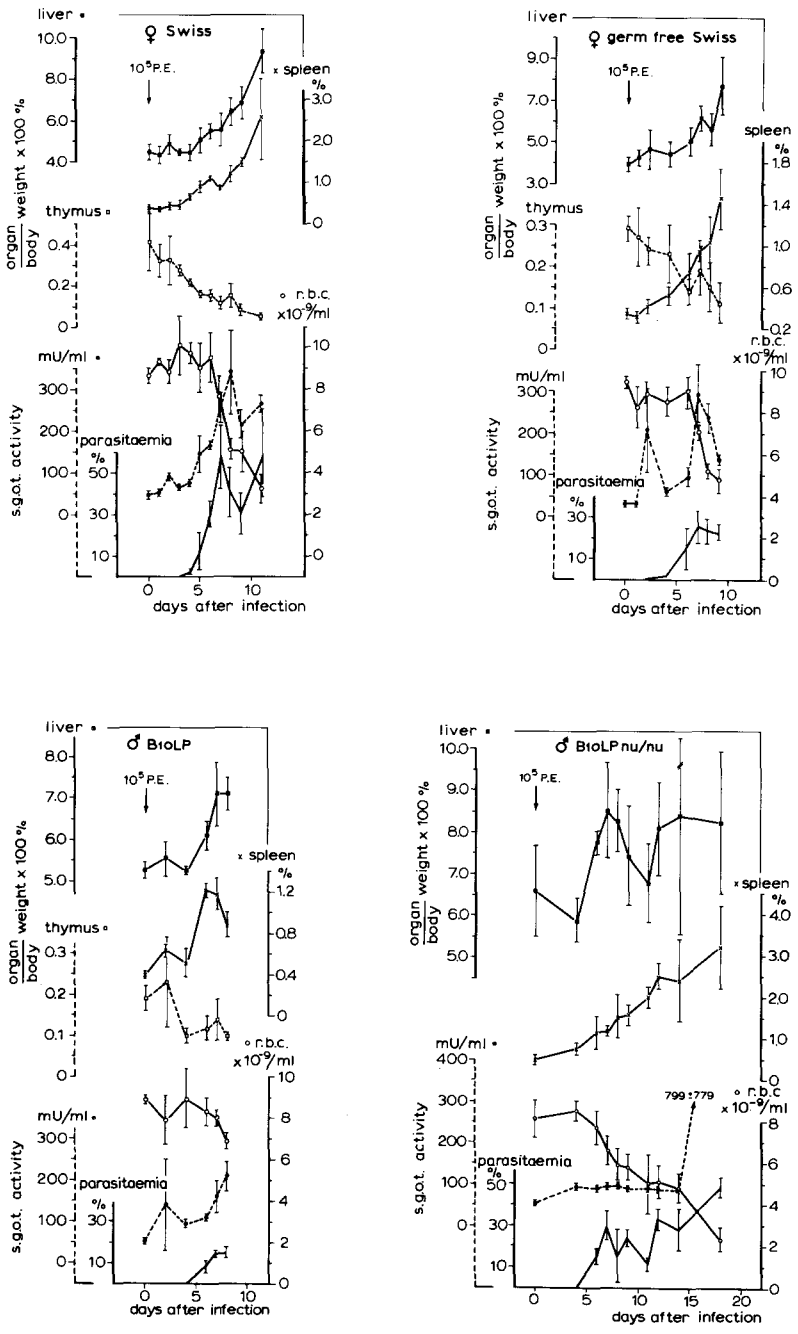

Fig. 2. Compilation of results in different mouse strains after infection with 10^5 P.E. For each mouse strain the course of the parasitaemia (———), the number of r.b.c./ml blood (—O—O—), the s.g.o.t. activity (●——●——●) and the percent organ-body changes for thymus (□——□——□), spleen (×—×—×), and liver (■—■—■) are depicted together

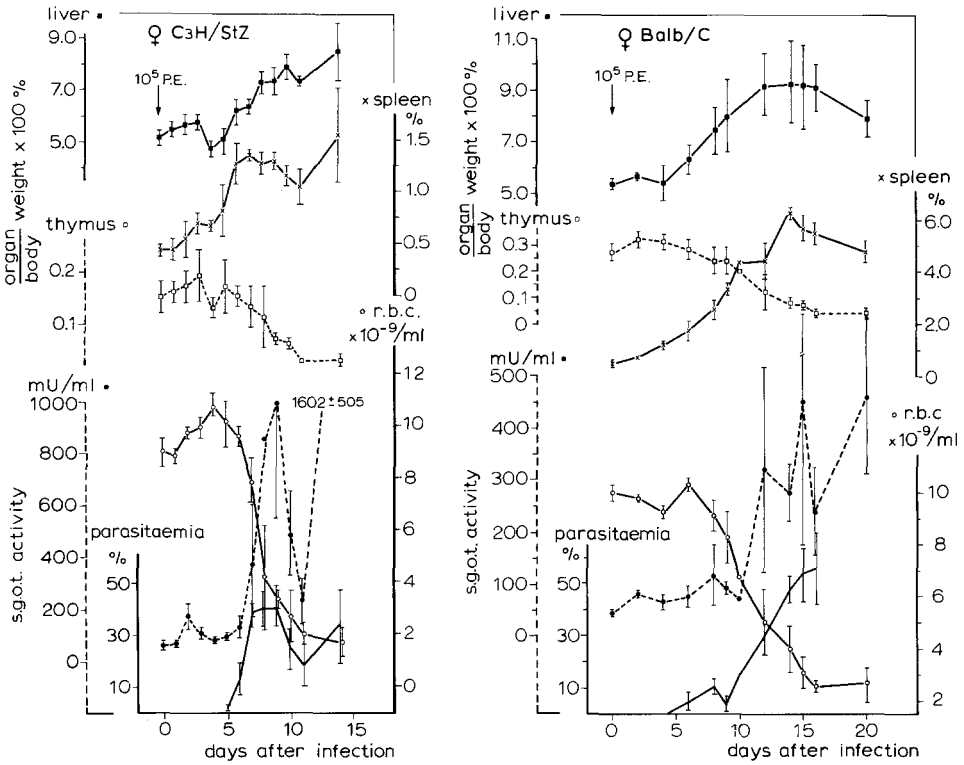

Fig. 3. See legend to Fig. 2

during a period of six days after inoculation can be noticed with a maximum number of r.b.c. approximately four days after infection. In the other combinations a more or less distinct, temporary decrease of the number of erythrocytes is obvious during this period. This initial loss of r.b.c. is most pronounced in Balb/c mice, and minimal in B10LP nu/nu, and B10LP mice. The definitive and progressive reduction in the number of red blood cells starts approximately six days after infection, and is independent of the parasite–mouse strain combination. The day on which a decrease of 50% of the original number of erythrocytes is observed characterized the strain specific progression of anaemia. This is found to be day 8–9 in C3H/StZ and Swiss mice, day 9–10 in germfree Swiss mice, day 12–13 in Balb/c mice and day 14 after infection in B10LP nu/nu mice. Data from recent additional experiments with B10LP mice (not shown in Fig. 2) revealed that a 50% reduction occurred after day 10 of the infection.

Anaemia may become very severe, and as few as 10% of the original number of erythrocytes may be present for several days before the animal succumbs. On the other hand, numerous B10LP mice, and also other strains (except Balb/c and B10LP nu/nu), are observed to die before a distinct reduction in the number of erythrocytes is noted.

The Serum Glutamic Oxalacetic Transaminase (s.g.o.t.) Activity

In controls the mean value of s.g.o.t. activity ranges from 43 mU/ml in Balb/c and Swiss mice to 58 mU/ml in germfree Swiss and C3H/StZ mice (Table 1).

After infection a more or less distinct and transient increase of s.g.o.t. activity is observed over a period of approximately four days with a peak value after two days. The magnitude of this peak activity is strain specific (Figs. 2, 3; for ratios see Table 1). In all but the B10LP nu/nu strain a second peak of s.g.o.t. activity is observed in connection with oxyphilic peak parasitaemia. This also holds true for the B10LP strain as was verified in additional experiments not indicated in the figure. The magnitude of s.g.o.t. activity at peak parasitaemia is also strongly strain specific (for ratios see Table 1). In C3H/StZ, Swiss and B10LP mice the second peak of s.g.o.t. activity does not coincide with peak parasitaemia but one day later. When polychromatocytosis develops after the oxyphilic peak and crisis and parasitaemia increases again, the s.g.o.t. activity rises too, and extremely high values can be found.

In B10LP nu/nu mice a slight but gradually increasing s.g.o.t. activity is observed between day 4–10. Thereafter a dramatic increase in activity can be observed in part of the mice. In additional experiments the effect of haemolysis on s.g.o.t. activity was analysed. No quantitative relation was obtained between serum haemoglobin concentration and s.g.o.t. activity in samples of control and infected mice. Lysates of uninfected cells exhibit low s.g.o.t. activity whereas that of infected cells was negligible. Addition of the lysates to s.g.o.t. containing samples did not specifically influence the s.g.o.t. determinations.

Body and Organ Weight

During the course of the infection a reduction in body weight (Fig. 4) is observed in almost all strains. Germfree Swiss and B10LP nu/nu mice are exceptions, probably because of the inevitable heterogeneity of these groups (see Materials and Methods). Whereas in Balb/c mice a continuous decrease in body weight is observed in Swiss, C3H/StZ, and B10LP mice body weights decrease especially near oxyphilic peak parasitaemia. From that time on mice look severely ill; they become progressively inert, develop white ears, nose and tail, a ruffled fur, and curved back. The longer they survive the higher the role of infectious complications such as diarrhoea, a wet

Table 1

	Ox. peak inf. $\bar{p} \pm$ s.d. (%)	Survival time m.s.t. $\pm$ s.d. (days)	S.g.o.t. activity (mU/ml)		
			Controls ($\bar{x} \pm$ s.d.)	Ratios of	
				d. 2 p.i. / controls	Ox. peak inf. / controls
♀ Balb/c	11 ± 3	23.5 ± 5.3	43 ± 6	1.9	2.7
♂ B10LP	15 ± 3	7.8 ± 0.9	50 ± 11	2.8	4.2
♀ germfree Swiss	25 ± 8	25.2 ± 10.2	58 ± 6	3.6	5.0
♂ B10LP nu/nu	30 ± 7	29.9 ± 6.6	55 ± 7		1.6
♀ C3H/StZ	40 ± 7	11.7 ± 3.2	58 ± 18	2.9	14.7
♀ Swiss	58 ± 16	12.8 ± 5.0	43 ± 12	2.1	6.0

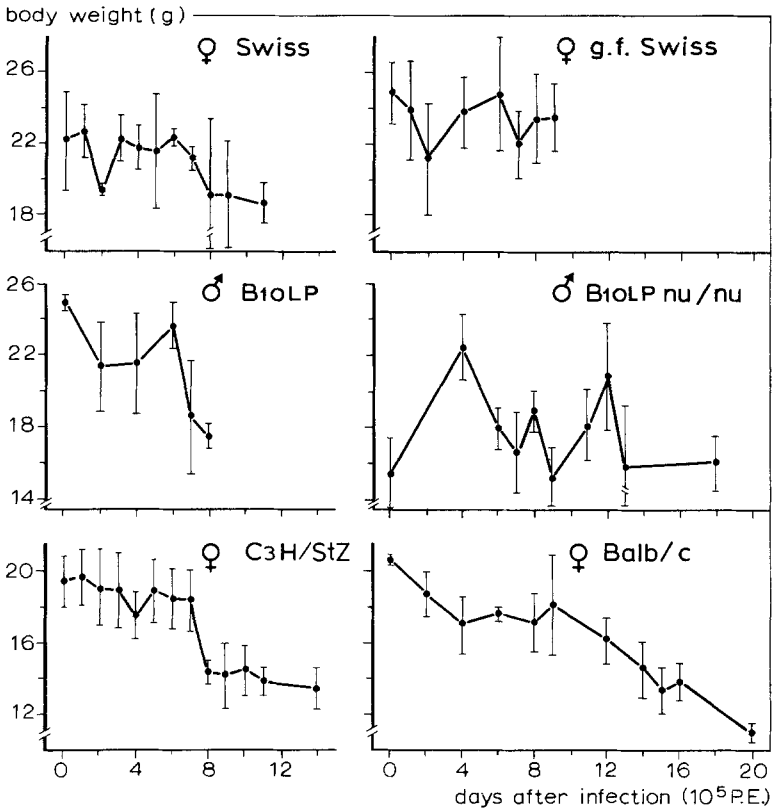

Fig. 4. Changes in body weight in 6 different mouse strains after infection with 10^5 P.E.

nose, or infected eyes. Facilitation of access to food pellets and drinking water neither improve their physical conditions, nor prevent the syndromes.

Thymus

During the course of the disease thymus involution is common to all strains, except of course for the thymusless B10LP nu/nu mice. The involution is usually so intensive that during the later stages of the disease hardly any thymic tissue can be prepared accurately free of fat or other adjacent tissue for determination of the organ weight. The involution proceeds differently in the various strains of mice. In Swiss and germfree Swiss mice thymus weight decreases already early after infection. In Balb/c, C3H/StZ and B10LP mice a temporary increase in thymus weight is observed during the first days after infection. The subsequent and definite thymic involution starts on day 4 after infection in B10LP mice, and one day before or on the day of peak parasitaemia in C3H/StZ and Balb/c mice, respectively.

Spleen

All mouse strains exhibit an increase in spleen weight during the course of the disease, starting early after inoculation. The rate of increase in spleen weight is approximately the same in all strains. With the exception of Balb/c mice, spleen weight increases almost continuously throughout the observation period. Enlargement of the spleen is most impressive in B10LP nu/nu, Balb/c and germfree Swiss mice. On the other hand both the C3H/StZ and B10LP strain exhibit a distinct but transient decrease in spleen weight around oxyphilic peak parasitaemia. During this period only a few mice of these strains are able to develop polychromatocytosis. Mice of these strains which develop polychromatocytosis, however, always have higher spleen weights than mice without polychromatocytosis.

The colour of the spleen becomes brown and in later stages almost black. The change in colour is apparent by the end of the first week after infection. Pigment accumulation appears more intense as the spleen increases in size and weight.

The Liver

A more or less distinct but slight increase in liver weight is observed during the four days following inoculation. Thereafter, liver weight increases remarkably. In almost all instances this increase is gradual and uninterrupted, neither during oxyphilic peak parasitaemia nor crisis, except for the B10LP and B10LP nu/nu strains where the increase in liver weight may be arrested or even temporarily decreases during this period. By the end of the first week after infection the liver usually develops a brown and in later stages an almost black colour. During the second week of infection the surface of the liver frequently exhibits a patchy appearance.

Discussion

Mortality Patterns

In a well adapted *Plasmodium berghei*–mouse combination the mortality pattern exhibits strain specificity (Greenberg and Kendrick, 1957). From our results four types of mortality pattern can be distinguished: (a) deaths predominantly in a monophasic wave early during the second week (B10LP); (b) bimodal mortality with modes early during the second week and during the third week (Swiss). Since the Swiss strain was the only outbred strain used in these studies, it remains to be investigated whether genetic disparity may be responsible for this phenomenon; (c) pronounced mortality occurring intermediate between the first and second peak of the Swiss mice pattern (C3H/StZ); (d) predominantly late mortality, starting during a period where the Swiss strain exhibits the second wave or even later (B10LP nu/nu, Balb/c and germ-free Swiss).

The irregularly and slowly increasing cumulative late mortality among mice and strains that survive beyond the second/third week after infection may result from a complex of processes. In these severely diseased mice secondary infections and nutritional deficiencies may become fatal before the mice die of malaria. The great standard deviation of the mean survival time (Table 1) may reflect this situation, and also indicates the limited informational value of the mean survival time in these strains. This also holds true for Swiss mice in which the mean survival time does not

characterize the typical bimodal mortality (Greenberg and Kendrick, 1957). Our data are somewhat at variance to those of Greenberg et al. (e.g. 1953; Greenberg and Kendrick, 1957), who also characterized a *P. berghei* infection in Swiss, C3H/StZ, Balb/c and other strains. Deviating results may be due to differences in the *P. berghei* strain, the size of the inoculum, and the route of inoculation. The introduction of infected syngeneic donor animals for the preparation of the infectious inoculum in our series appears to be important.

In contrast to the above cited studies in our hands the mortality pattern of Swiss and C3H/StZ mice are essentially different, whereas an almost identical and bimodal mortality pattern was observed when exclusively infected Swiss mice served as donors. Thus, our improved discrimination of strain specific patterns argues against a generalized model characterized by a major assault at the end of the first week, followed by a cyclical series of attacks producing a series of modes of time of death, as proposed by Greenberg and Kendrick (1958). For this reason it is advisable to use only well adapted parasites in studies on parasite–host relationship.

Parasitaemia

A subpatent period of approximately four days, proliferation of parasites in oxyphilic mature erythrocytes until a peak parasitaemia on day 7–8, and followed by crisis are the common features of all parasite–mouse strain combinations studied. Comparable observations were made by Greenberg (1956) with other *Plasmodium berghei* mouse strain models. This might indicate that these features are not determined by host dependent, metabolic factors, e.g. availability and concentration of PABA, and the erythrocytic A.T.P. concentration. However, both the strain specific rate of parasitic proliferation, and the proportion of infected cells at peak parasitaemia (Table 1) appear to depend on genetically determined metabolic factors, as suggested for the erythrocytic A.T.P. level by Brewer and Powell (1965) and Brewer and Coan (1969). If limitation of peak parasitaemia and crisis are not induced by a shortage of metabolites necessary for parasitic proliferation, an immune response to parasitized oxyphilic erythrocytes may be considered, although the day of first peak parasitaemia and the start of crisis after inoculation vary with the number of P.E. inoculated (Eling and Jerusalem, 1977a). Our results do not indicate a causal relation between the magnitude of this peak infection and death during the second week, or with the mean survival time (Table 1), as discussed by Greenberg (1956). It therefore appears doubtful that early death is caused by toxaemia due to acute destruction of P.E. at peak infection and during crisis (Greenberg, 1956; Greenberg and Kendrick, 1957, 1958).

In our model survival of mice beyond the second week after inoculation always coincided with the occurrence of polychromatocytosis. It remains uncertain, however, whether increased survival is caused by the ability of the mouse to develop polychromatocytosis in time, or whether polychromatocytosis occurs in all mice, provided they survive long enough. Subsequently to crisis and development of polychromatocytosis the parasite switches host cell preference from mature oxyphilic to polychromatophilic erythrocytes and apparently regains the ability to proliferate. If indeed crisis is to be explained by an immunological response to infected oxyphilic erythrocytes, the invasion of and proliferation in polychromatophilic erythrocytes might indicate evasion of that response. In that case the observed persistence of small numbers of infected oxyphilic erythrocytes during polychromatocytosis, however,

remains unexplained. Mortality could then be explained by the inability to generate a response to infected polychromatophilic cells. In Swiss mice infected with *P. berghei* a severe immunodeficiency syndrome at this stage of the disease was described previously (Sengers et al., 1971a).

Since the congenitally athymic B10LP nu/nu mice do exhibit oxyphilic peak parasitaemia, crisis, polychromatocytosis and the concomitant switch in host cell preference of the parasites, the early immunological reactions would be independent of T-cells.

Erythrocyte Numbers

Changes in numbers of r.b.c. appear to take place in two phases. During the first six days following infection erythrocyte numbers transiently either increase (C3H/StZ and Swiss), or decrease (Balb/c and germfree Swiss) more or less distinctly, whereas the level remains constant in B10LP and B10LP nu/nu mice. The aetiology of these changes is still obscure. They may be caused by an increased water influx and efflux due to changes in vascular permeability, or by a shift of cells from and into erythrocytic reservoirs, e.g. the liver and spleen. Early variations in erythrocyte numbers are neither related to early and transient increases in s.g.o.t. activity (see next section), that might indicate certain early cellular lesions, nor to late pathophysiologic and histopathologic changes. This first phase of changes of r.b.c. numbers was not mentioned by previous workers (Kretschmar, 1961; Jerusalem, 1964; Greenberg, 1956), perhaps because they worked with an infectious inoculum consisting of at least 10 times more P.E., thus accelerating the sequence of events and masking early reactions.

The second phase is characterized by progressive anaemia which starts approximately six days after inoculation in all mouse strain–*P. berghei* combinations, i.e. before oxyphilic peak infection. Progressive loss of r.b.c. appears to be independent of the proportion of infected erythrocytes, as indicated by a highly varying magnitude of the first peak of the parasitaemia. For this reason the destruction of erythrocytes through repeated movements of the parasite (Lawson, 1920) or through the action of malaria toxin (Brown, 1912, 1913; Greenberg, 1956) are unsatisfactory explanations (Greenberg, 1958; Kretschmar, 1961). Jerusalem (1964) and Zuckerman (1966) discussed a possible role of auto-antibodies. Since in congenitally athymic B10LP nu/nu mice anaemia occurs to the same extent, an anti-erythrocyte auto-antibody could have to be synthesized in the absence of T-helper cell activity, and therefore should consist mostly of IgM (Jerusalem and Jap, 1977). Furthermore anaemia also develops to the same extent in splenectomized mice (Kretschmar and Jerusalem, 1963; Topley et al., 1970) implying that the spleen is not involved either directly or indirectly in a hypothetical auto-immune response.

Whereas the beginning of the definitive progressive anaemia on day 6 is almost independent of the mouse strain, the rate of fall in cell count is strain specific. The rate of blood loss could be a measure of the intensity of the (immuno-) pathological reaction. Except for the B10LP strain this is illustrated by the correlation between the increased mean survival time (Table 1), and a decreased rate of fall in cell count. The B10LP mice, however, combine the shortest mean survival time (Table 1) with a reduction in the number of erythrocytes to 50% occurring no earlier than 10 days after inoculation.

On the other hand, a transient increase in the number of erythrocytes during the first six days after inoculation is followed by a more rapid fall in cell counts, as

compared to an unchanged or transiently decreased number of erythrocytes during the early period. The operational mechanism, however, is obscure. Since the actual number of erythrocytes is the result of a balance between sequestration and destruction and production and release from reservoirs or erythropoietic tissues, respectively, these processes need to be assessed separately. Since mice can survive despite a very severe anaemia while others succumb before distinct anaemia has developed, anaemia is not likely to be a primary cause of death. We refer again to auto-antibodies reported to play an aetiologic role in the loss of r.b.c. Since anaemia may well be related to the mean survival time, but is not primarily causative, cross-reactions of antibodies with other tissue antigens, and/or non-specific trapping of immune complexes could be factors involved in the pathogenesis of a fatal course.

The s.g.o.t. Activity

A transient increase in s.g.o.t. activity during the first four days after infection was depicted, but its transient character not discussed by Sadun et al. (1966). According to their results, activity of the enzymes increased when the number of P.E. in the inoculum was increased. Therefore, as mentioned for early variations of erythrocyte numbers with large inocula, the early delicate changes in s.g.o.t. activity are masked due to a dose dependence of the response. It can be stated that: (a) the phenomenon of s.g.o.t. increase is the earliest pathological change observed so far. The mechanism responsible for the liberation of s.g.o.t. may either be interrupted or repaired after four days, whereas the transient character implies a rather rapid turnover of liberated s.g.o.t. in the blood; (b) the phenomenon occurs before P.E. are found in thin smears; (c) although quantitatively strain dependent the time dependent course of the process is comparable in all strains tested; (d) the magnitude of the s.g.o.t. activity during the four days after inoculation cannot be taken as a marker for subsequent changes in any of the parameters measured; (e) no quantitative correlation is observed with the s.g.o.t. activity during the second phase, e.g. at peak parasitaemia (Table 1).

The time dependent correlation is clearly indicated by results according to which peak parasitaemia is always noticed on the same day or one day earlier (C3H/StZ, Swiss and B10LP) than the peak of s.g.o.t. activity. Furthermore, a rapid proliferation of parasites towards oxyphilic peak parasitaemia is closely followed by a rapid increase in s.g.o.t. activity, and a reduction in the proportion of infected erythrocytes is correlated with a decrease in s.g.o.t. activity. Moreover, in instances of rising polychromatocytosis and renewed proliferation of parasites s.g.o.t. activity increases correspondingly. The absence of both a correlation between s.g.o.t. activity and serum haemoglobulin levels, and an effect of lysates of infected erythrocytes on s.g.o.t. determinations in positive samples indicate that the destruction of erythrocytic hosts associated with the proliferation of parasites apparently does not account for changes in s.g.o.t. activity. In addition, another fact argues against haemolysis as a suggested causal mechanism of increased s.g.o.t. activity (Einheber et al., 1967; Maegraith, 1966). During crisis, when a rapid loss of r.b.c. is observed, the s.g.o.t. activity decreases. Generally, variations in the rate of developing anaemia are not paralleled by changes in s.g.o.t. activity.

Supposing that changes in parasitic proliferation are at least to some extent influenced by an antibody mediated immune response, the same or a corresponding reaction may be considered to be responsible for the increased release of s.g.o.t. Since in most instances of elevated s.g.o.t. levels the liver appears to be involved

(Sadun et al., 1966; Maegraith, 1966; Martin et al., 1966) this organ may possibly be the target of immune reactions discussed in the previous section.

Remarkably enough, strains with comparatively long survival and death only after crisis (B10LP nu/nu and Balb/c) also exhibit low s.g.o.t. activity during the first two weeks after inoculation. In B10LP nu/nu oxyphilic peak parasitaemia is not reflected by peak values of s.g.o.t. activity which remains at a rather constantly elevated level. Furthermore low s.g.o.t. activity is not in every case prognostic of increased survival, as shown with germfree Swiss mice. Although peak values of s.g.o.t. activity around oxyphilic peak infection and persisting high values in the period after crisis apparently indicate a severe pathological state, the absolute measures of the activity of this enzyme cannot be taken as a marker of an immediately fatal situation.

Changes in Body and Organ Weight

The decrease in body weight during the course of the disease is a rather constant feature (Fig. 4). Since most mice appear severely ill by the end of the first week, reduced water and food intake may be causative, although features of starvation and/or dehydration are hardly distinguishable from some other alterations. For this reason, and to eliminate deviations due to inhomogeneity of the groups (germfree Swiss, B10LP nu/nu) the organ weights are expressed as a percentage of body weight. In addition, the presentation of results in an organ/body weight relation provides more detailed information (Jerusalem, 1977). Furthermore, the same characteristics of changes in organ weights were observed when absolute values were compared with calculated percentages.

Thymus

Thymus involution previously described by Singer (1954) appears to be a common feature in all *Plasmodium berghei* mouse models. There are indications that the functional lesion of the thymus precedes the morphologic one, since the activity of ATP: Thymidin-5′-phosphotransferase decreases rapidly already after the 3rd day of infection (Jerusalem, 1965; Eling and Jerusalem, 1968). The accidental involution of the thymus raises questions concerning both its aetiology, and its relevance to the immunocompetence of the host. Wereas adult thymectomy has no immediate deleterious effect on immune response of the host (Miller, 1966) a *Plasmodium berghei* infection in mice severely inhibits T-cell-mediated reactions (Sengers et al., 1971). Therefore, impairment of T-cell function appears to be more generalized and includes the circulating and resident compartment of the pool of T-lymphocytes. According to our results the characteristics of the involutionary process are strain specific. A temporary increase in thymus weight during the first week after inoculation (Balb/c, C3H/StZ and B10LP) remains unexplained. It may depend on an increased influx of precursor cells secondary to peripheral antigenic stimulation (Micklem et al., 1972), or changes of the kinetics within the thymus. Although the thymus is considered to be a "primary" organ, functioning independently of external influences, it appears to respond to peripheral stimuli in various ways (Hagmann et al., 1977). In all strains of mice tested thymic involution is evident on the day of peak parasitaemia, but the regression is apparently not associated with the rate of parasitic proliferation, and the number of infected r.b.c. The activity of s.g.o.t. neither

correlates with the onset of the regressive process, nor with the rate of involution. Therefore, a simple causative factor as common denominator (e.g. a toxin triggering a variety of pathological processes, such as s.g.o.t. release through cell damage, anaemia, and thymus involution) appears unlikely. Compared to the parent B10LP strain the activity of s.g.o.t. in athymic B10LP nu/nu around oxyphilic peak infection is only slightly increased, despite an apparently normal proliferation of parasites.

These findings suggest thymus-derived (T-)cells to be involved in processes which take effect, e.g. in the liberation of s.g.o.t. Generally, T-cells play a crucial role in establishing specific immunity, either in humoral responses through triggering B-cells to synthesize IgG or in cell-mediated cytotoxicity by instructing ("arming") macrophages. Since the lack of T-cell functions largely inhibits specific immune reactions, the question arises whether increased liberation of s.g.o.t. results from manifestations of immune mechanisms, causing immunologic injury. Adverse effects of immune reactions are well documented, as well as the efficiency of immuno-suppression in inhibition of immunopathologic lesions. Theoretically the extended mean survival time of athymic B10LP nu/nu mice as compared with the parent strain could result from the absence of harmful responses mediated by T-cells. The interacting mechanisms, however, may be more complex, since increased survival times were also observed in the normal thymus-bearing Balb/c, and particularly in the germfree Swiss strain. The effect of internal and external factors like alterations of endocrine functions as described in athymic mice (Pierpaoli and Sorkin, 1972) or an environmental deficiency state (germfree conditions) on the survival time are largely unexplored.

The Spleen

An increase in spleen size and weight is a common feature of *Plasmodium berghei*—mouse combinations, at least in the initial phase. Thereafter, around oxyphilic peak parasitaemia either the spleen weight increases further, which is associated with both the development of polychromatocytosis and prolonged survival or it decreases. In the latter instances polychromatocytosis is absent, and mortality occurs earlier. In addition to hyperplasia of elements of the R.E.S., assumedly linked to uptake of parasite derived materials (e.g. pigment), the spleen is involved in two other processes of cellular kinetics. First, the initial proliferative response of the splenic white pulp (Jerusalem, 1965) suggests an immune reaction to parasitic antigens. In both CF mice (Singer, 1954), and N.M.R.I. mice (Kretschmar and Jerusalem, 1963) the total mass of splenic lymphatic tissue subsequently decreases progressively, despite enlargement of the spleen. Referring to accidental thymic involution discussed above, severe reduction of the white pulp may not only indicate a more generalized attack on the T-cell system, but also a collapse of the whole of splenic lymphatic tissue.

Secondly the absolute increase in spleen weight results particularly, if not exclusively, from proliferation of the erythropoietic tissue (Kretschmar and Jerusalem, 1963). The changes in spleen weight could therefore be explained in terms of the ability of the mice to mount an erythropoietic response. Indeed, mice that survive for a longer period exhibit a gradual increase in erythropoietic tissue in the spleen, and polychromatocytosis. In contrast, mice or mouse strains which die around oxyphilic peak infection lack polychromatocytosis, apparently because of inhibited extramedullary erythropoiesis, and the weight of the spleen even decreases due to the reduction of the lymphatic compartment. Although intensified

erythropoiesis, and accompanying polychromatocytosis appear directly related to increased survival, it cannot be excluded with certainty that the proliferative response became manifest only due to longer survival.

The increase in spleen weight during the first week after inoculation is approximately the same in all mouse strains, suggesting that infection as such triggers the proliferative processes, rather than a correlation with the magnitude of the parasitaemia, s.g.o.t. activity, or thymic involution respectively. Since erythropoiesis predominantly determines the changes in spleen weight, the alterations of the white pulp and their implications in the disease may be masked. Nevertheless, thymic involution and the host's inability to mount an immune response to a second antigen (Sengers and Jerusalem, 1971) suggest lesions of the defence mechanism to be of primary importance (Moran et al., 1973).

The Liver

The involvement of the liver in human and experimental malaria (Maegraith, 1968) as well as in rodent malaria (Sadun et al., 1965, 1966) is amply documented. In our series initial variations in liver weight were transient, and paralleled by comparable changes in s.g.o.t. activity. However, no obvious correlation can be made with the pattern and magnitude of the s.g.o.t. activity beyond the period of four days. Although the macroscopically patchy appearance of the liver obviously reflects necrotic areas, it remains to be investigated whether high s.g.o.t. activity results from these focal hepatic lesions. Dilatation of sinusoids due to congestion with parasitized erythrocytes and other blood cells in addition to ongoing phagocytosis of debris by the Kupffer cells, or a generalized injury like cloudy swelling or hydropic degeneration, respectively, may explain the increase in liver weight and change of colour.

Conclusions

The present study revealed *P. berghei* malaria to produce a number of common features in the various mouse strains. The extent to which these features are expressed, however, is strain specific. The relevance of these findings to human malaria, i.e. in genetically divergent hosts, is obvious. Our results further indicate that several organs, and organ systems are severely involved. A detailed histopathologic analysis of these organs will be necessary to elucidate possible interrelationships of processes in the organs.

Acknowledgement. The skilled technical assistance of Mrs. M.v. Zon-Cremers, J. Koekkoek and J. Reynders is gratefully acknowledged. We are indebted to Messrs. J. Reitsma, G. Poelen and M. Faassen of the central animal laboratory (Head: Dr. W.v.d. Gulden) for their biotechnical collaboration and care of the animals and to M.L. Weiss for reading the manuscript.

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Received April 21, 1977