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Level of Complement Activity and Components C1, C4, C2, and C3 in Complement Response to Bacterial Challenge in Malnourished Rats

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In experimentally induced malnutrition in rats, there was no significant difference between the measured level of complement activity of the classical pathway (50% hemolytic complement [CH_{50}]) and that of the alternative pathway (ACH_{50}), although the levels of complement components C1, C4, C2, and C3 were depressed significantly. The complement activity showed a temporary elevation with a peak at 2 or 3 days after bacterial challenge with *Staphylococcus aureus* in rats, and we call this the complement response. After 3 days, CH_{50} and C3 in the malnourished rats and ACH_{50}, CH_{50}, and C3 in the well-nourished rats showed a significant increase, and C1, C4, and C2 in both groups tended to elevate. On the basis of these observations, the significance of the elevation of C3 in the complement response to bacterial infection showed a strong influence by enhancing the activation of both the classical and the alternative pathways, since C3 is known to be the junction of both complement pathways. In this way, C3 responded to an earlier stage than did the other components and may contribute to maintaining the body defense system against infection.

It has been reported by many investigators that the host defense mechanisms, i.e., cell-mediated immunity and humoral immunity, are depressed in a malnourished state (2-5, 18) and that malnutrition is considered to be associated with increased susceptibility to infection.

We have been analyzing host resistance factors, especially the complement system and the cell-mediated immunity system, in malnourished rats and in malnourished, infected rats.

In a previous experiment, we observed a temporary elevation of complement activity 2 or 3 days after experimental infection in both malnourished and well-nourished rats. We call this phenomenon the complement response (17).

During this complement response, increased incorporation of [^{14}C]leucine into the serum fraction, which combined with the antigen-antibody complex, was shown in both the malnourished and well-nourished rats (M. Sakamoto et al., unpublished data).

Based on these observations, the level of ACH_{50} (50% hemolytic complement activity of the alternative pathway), CH_{50} (50% hemolytic complement activity of the classical pathway), and the individual complement components, C1, C4, C2, and C3, in the complement response stage was measured.

MATERIALS AND METHODS

Animals. Five-week-old male Sprague Dawley (specific-pathogen-free [SPF]) rats, purchased from Shizuoka Farm, Shizuoka, Japan, averaging 80 g in weight, were used for this experiment.

The animals were housed individually in stainless-steel cages in an air-contitioned room at 24 ± 2°C and 50 ± 10% humidity with lighting regulated to provide 12-h intervals of light and darkness.

Diet. The animals were divided into two groups: the 18% casein diet group (the 18% group) and the 0.5% casein diet group (the 0.5% group). Each group of rats was given a limited diet: 20 g of 18% protein per day per rat (8) or 15 g of 0.5% protein per day per rat. The rats were fed three days per week throughout the experiment. Water was available to the rats at all times. The composition of the diets has been previously described (12, 16).

Infection. The rats were infected with *Staphylococcus aureus* 226. A 0.20-ml amount of 2×10^9 organisms per ml or 8×10^9 organisms per ml of phosphate-buffered saline was injected intradermally into the abdominal skin of the rats after 8 weeks of feeding them with the 0.5% protein diet or the 18% protein diet, respectively. The grade of inflammation was observed in the back sites when the skin of the injected regions was flayed. The inflammatory reaction due to 4×10^8 *S. aureus* organisms per 0.20 ml in the malnourished rats was almost of the same magnitude as the inflammatory reaction due to 1.6×10^9 *S. aureus* organisms in the well-nourished rats, giving abscesses of a diameter of ca. 4 mm surrounded with hyperemia.

Complement measurements. To monitor the complement systems, we measured the CH_{50}. To take these measurements, we followed the method of Mayer (6), requiring incubation at 20°C for 60 min (15) and employing sheep erythrocytes sensitized with

an optimal amount of rabbit antibody.

To measure the ACH_{50}, we used hemolytic activity of serum to rabbit erythrocytes in ethylene glycol-bis(β-aminoethyl ether)-*N,N*-tetraacetic acid buffer (1, 13).

The individual components, i.e., C1, C4, and C2, were measured by their immune hemolytic activity by using the microtiter method. Guinea pig complement components and intermediate cells were prepared in our laboratory and measured by the method described by Nelson et al. (9). The measurement of C1 started from a 200 × dilution of the original serum. Component C3 was measured by immune adherence reactivity by using the method described by Nishioka (10). Care was taken to titrate components as soon as possible after the serum sample was thawed (7).

Experiment. Sixty male rats were divided into two groups. The control group, 20 rats which received the 18% protein diet, was considered to be the well-nourished group, and the other group, 40 rats which received the 0.5% protein diet for 8 weeks, was designated the malnourished group.

The state of malnutrition was confirmed by comparing measurements of the body weight of the rats and the hematological changes with standards described previously (16).

The blood was withdrawn from the tail vein of each of the rats in the 18% group and from the axilla in each of the rats of the 0.5% group. The blood was centrifuged at 4°C and 3,000 rpm for 10 min and stored at −80°C immediately after separation of the serum, and all the complement components were measured simultaneously with a known control serum as an internal standard.

RESULTS

Confirmation of the nutritional state. The nutritional state of the rats was confirmed by comparing body weight and hematological observations with standards set in our previous paper (16). The average body weight of the rats fed 0.5% protein for 8 weeks was 63.7 ± 1.22 g, and that of the rats fed 18% protein was 350.0 ± 3.75 g.

The hematological observations showed changes in the malnourished stage and in the normal stage similar to previous results (16).

Changes in complement level and the components in the malnourished state. In comparing the effect on the CH_{50} of different nutritional factors, the CH_{50} of the 0.5% group had a lower titer than that of the 18% group after 8 weeks of feeding, but the differences were not significant (Table 1). This tendency of the complement levels of the two groups to be about the same confirmed previous data.

The ACH_{50} level was also lower, and not statistically significant in the 0.5% group as in the 18% group (Table 1). On the other hand, components C1, C4, C2, and C3 decreased more significantly ($P < 0.05$) in the 0.5% group than in the 18% group.

Changes in the complement systems and the components in the complement response 3 days after infection. The complement titers 3 days after infection are shown in Table 2. Whether the rats were in the malnourished or normal nutritional state during experimental infection, the complement titer (CH_{50}) elevated significantly in our previous experiment (17). In this experiment, elevation of the CH_{50} in both the 0.5% and 18% groups confirmed those results. There was a more significant elevation of complement in the infected groups than in the noninfected groups in both the 0.5% ($P < 0.01$) and 18% ($P < 0.05$) groups.

The level of the individual components C1, C4, C2, and C3 in the infected group showed a tendency to increase. The C3 titer ($P < 0.05$), especially, was much higher than that of the noninfected group.

In the 18% group, after the infection the ACH_{50} titer also increased significantly ($P < 0.05$). When the host was infected in the normal nutritional state, CH_{50}, ACH_{50}, and the C3 component levels increased remarkably ($P < 0.05$). These results indicate that all the complement systems, i.e., the classical pathway, the alternative pathway, and among the complement components, C3 specially, are actively enhanced by infection.

In the 0.5% group, the CH_{50} had a much more significantly higher titer ($P < 0.01$) than that of the control group, but the ACH_{50} did not show any marked difference. Of the individual components, only C3 showed a significantly higher

TABLE 1. *Complement systems and components in malnourished rats*[a]

Diet	Titer (mean ± standard error)					
	CH_{50}	C1[b]	C4[b]	C2[b]	C3[c]	ACH_{50}
18%	108 ± 6.7	4,800 ± 940	419 ± 93	157 ± 30	29,500 ± 7,200	4.2 ± 0.22
0.5%	95 ± 7.0	2,600 ± 260[d]	147 ± 19[d]	66 ± 12[d]	5,900 ± 1,100[d]	3.7 ± 0.51

[a] Rats were fed either the 18% protein diet or the 0.5% protein diet for 8 weeks. Ten male rats were used in each group.

[b] End titer of serum dilution giving ca. 50% hemolysis starting from undiluted sera (C4, C2) or a 1/200 dilution (C1).

[c] End titer of serum dilution giving ca. a 2+ immune adherence hemagglutination pattern.

[d] $P < 0.05$; significant difference between data of the 18% group and those of the 0.5% protein group.

titer ($P < 0.05$); C1, C4, and C2 did not show any significant difference during infection.

Changes in the complement systems 14 days after infection. In the 18% group with mild inflammation, 14 days after the infection, the complement system did not show any significant changes (Table 3). When the infected rats were in the malnourished state, even if they maintained the inflammatory state 14 days after infection, complement elevation was not observed. These rats died within a few days. The rats fed the 0.5% protein diet for 10 weeks became more severely malnourished, and on week 11 started to die because of severe malnutrition. On week 10 of the 0.5% protein diet, the complement activity (CH_{50}) showed a significantly lower titer, but the level of the ACH_{50} was still maintained.

DISCUSSION

When the infected rats were in the malnourished state or in the normal state, complement activity temporarily elevated to a peak at 2 or 3 days after the onset of infection and, thereafter returned to its former level (17). We call this phenomenon the complement response.

In the complement response, the incorporation of labeled amino acid ([^{14}C]leucine) into serum fractions combined with the antigen-antibody complex was remarkably high in infected rats, especially in the malnourished state (M. Sakamoto, et al., unpublished data). This can be explained by a de novo synthesis of the complement enhanced by infection, especially in malnourished rats. In this report, the changes in the complement systems, i.e., the classical pathway, the alternative pathway, and the individual components C1, C4, C2, and C3, of the complement response in the malnourished rats were investigated. The control rats were fed an 18% casein protein diet and the malnourished rats were fed a 0.5% casein protein diet for 8 weeks. The nutritional states were confirmed by changes in the body weight and in hematology. After we induced malnutrition in the rats, *S. aureus* 226 was introduced intradermally into these rats and into those of the control group. The levels of CH_{50}, ACH_{50}, and C1, C4, C2, and C3 were measured 3 and 14 days after infection.

The nutritional factors in the 0.5% group did not significantly depress the CH_{50} and ACH_{50} levels compared to those of the 18% group, but the nutritional factors greatly reduced the levels of C1, C4, C2 and C3 in the 0.5% group.

TABLE 2. *Complement systems and components of the complement response 3 days after S. aureus 226 infection in malnourished rats*[a]

Diet	Infection	Titer (mean ± standard error)					
		CH_{50}	C1[b]	C4[b]	C2[b]	C3[c]	ACH_{50}
18%	No	108 ± 6.7	4,800 ± 940	419 ± 93	157 ± 30	29,500 ± 7,200	4.2 ± 0.22
	Yes	136 ± 7.2[d]	4,700 ± 500	547 ± 68	227 ± 58	240,700 ± 88,600[d]	7.7 ± 0.59[d]
0.5%	No	95 ± 7.0	2,600 ± 260	147 ± 19	66 ± 12	5,900 ± 1,100	3.7 ± 0.51
	Yes	139 ± 1.3[e]	3,200 ± 460	192 ± 50	110 ± 56	33,200 ± 11,000[d]	4.6 ± 0.30

[a] Ten male rats were in each group and were fed 18% and 0.5% protein diets for 8 weeks.

[b] End titer of serum dilution giving ca. 50% hemolysis starting from undiluted sera (C4, C2) or a 1/200 dilution (C1).

[c] End titer of serum dilution giving ca. a 2+ immune adherence hemagglutination pattern.

[d] $P < 0.05$; significant difference between data of the uninfected group and those of the infected groups.

[e] $P < 0.01$; significant difference between data of the uninfected group and those of the infected group.

TABLE 3. *Complement systems and components in malnourished rats 14 days after S. aureus infection*

Diet	Infection[a]	Titer (mean ± standard error)					
		CH_{50}	C1[b]	C4[b]	C2[b]	C3[c]	ACH_{50}
18%	No	141 ± 6.6	4,200 ± 920	151 ± 26	93 ± 21	15,100 ± 3,200	4.0 ± 0.12
	Yes	138 ± 2.6	2,300 ± 250	186 ± 25	121 ± 29	17,700 ± 3,200	3.8 ± 0.12
0.5%	No	90 ± 9.1[d]	2,500 ± 300	70 ± 6	53 ± 10	11,200 ± 3,200	3.4 ± 0.28
	Yes	95 ± 12.7	2,800 ± 590	73 ± 15	40 ± 7	12,700 ± 2,800	3.2 ± 0.25

[a] Infection with *S. aureus* 226; *strain*, 1.6×10^9 organisms for the rats in the 18% group and 4×10^8 organisms for the rats in the 0.5% group.

[b] End titer of serum dilution giving ca. 50% hemolysis starting from undiluted sera (C4, C2) or a 1/200 dilution (C1).

[c] End titer of serum dilution giving ca. a 2+ immune adherence hemagglutination pattern.

[d] $P < 0.05$; significant difference between the data of the 18% group and those of the 0.5% group in the uninfected group.

In malnourished rats, depressed antibody formation has been reported (3). Our observation is that the CH_{50} and ACH_{50} showed a lower titer but were not depressed significantly but that the complement components C1, C4, C2, and C3 were depressed significantly in the malnourished rats. These facts indicate that complement activity was not depressed significantly in the malnourished state, although there were other depressed immunological markers. The alternative pathway is probably working to maintain the complement activity during the malnourished state.

During infection, the complement titers through the classical pathway and through the alternative pathway increased in both the malnourished and the well-nourished control groups; CH_{50} in the malnourished group showed a remarkably higher titer after bacterial infection. It should be mentioned here that a higher grade of complement response and a higher rate of labeled amino acid incorporation into the de novo-synthesized serum protein exist in malnourished states after bacterial infection.

Of the individual components, C3 increased significantly. When the host had an infection, component C3 showed a strong influence in increasing the complement activity by enhancing activation of both the classical and alternative pathways, since C3 is known to be the junction of both complement pathways. In this way, C3 responded at a stage earlier than the other components and may contribute to maintaining the body defense system against infection. The earlier response of complement C3 than that of C1, C4 or C2 when the host had a bacterial infection is in line with the observation that, phylogenetically and ontogenetically, the C3 system appears earlier (11) and plays an important role in the body defense mechanism, combining the humoral and cellular immune systems. Therefore, in the malnourished state, especially during infection, it will be noteworthy to see the effect of a supplement of exogenous C3 protein or an induction of an increased level of C3 by the various procedures suggested by other investigators (19) for the purpose of enhancing the body defense mechanisms.

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