Lymphocyte Reaction to Antigen Stimulation in Protein Deficient Mice

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INTRODUCTION

Some functional disorders, such as increased susceptibility to certain types of infection[1-3] and poor immune response to antigen stimulation[4-6], have been reported in protein deficient animals. Recently, depressed cellular immune responses as measured by reduced rates of phytohemagglutinin (PHA) response[7-9] and sheep erythrocytes (E)-rosette formation[10] of lymphocytes have been found in human protein calorie malnutrition. In the previous report, we presented evidence indicating a marked involution of the lymphoid organs, particularly of thymus, and little change in adrenals and gonads in protein deficient mice[11]. In the present experiment, to further investigate the lymphocyte reaction in protein deficient mice, we have evaluated the fast green histone content of the lymphocyte nuclei and percentage frequency of the large pyroninophilic cells and plasma cells in the lymph node after antigen stimulation.

MATERIALS AND METHODS

Mice (CF#1) of forty five days old and of both sexes were fed the protein-free diet (0% casein) or the regular diet (25% casein), as previously described[11]. The mice were nursed normally, weaned at 21 days of age, and then maintained on a soild NMF diet (Oriental Yeast Mfg. Ltd., Tokyo) for 24 days, until 45 days of age. Thereafter, the experimental mice were fed the protein free diet for 14 days while the control mice were maintained on the regular diet, both for about the same period. To investigate the lymphocyte reaction, 0.1 ml of typhoid-paratyphoid vaccine (Takeda), or bovine gamma globulin (BGG, Nakarai), emulsified in an equal amount of compelte Freund's adjuvant (Difco) was subcutaneously injected into the hind foot pad of both the control and the experimental mice. Thirty minutes after the antigen injection, lymphocytes in the popliteal lymph nodes of these mice were smeared on slides and then stained with fast green FCF (Chroma) for

histone detection, according to the method of Alfert and Geschwind[12]. The content of fast green histone in the small lymphocyte nuclei was measured at 635 nm with a microspectrophotometer (Olympus MSP-AIV), according to the method described elsewhere[13-15]. The amount was expressed in arbitrary units. Female mice fed a regular diet, and those fed a protein-free diet at 45 days of age for 17 or 21 days were given antigen injections 3 or 7 days before killing at 62 or 66 days of age. The organ weight and total number of nucleated cells in the popliteal lymph nodes of those mice at 3 and 7 days after the antigen injection were estimated by the method of Awaya[16]. Male mice fed a regular diet, and those fed a protein-free diet at 45 days of age for 21 days were given antigen injections 7 days before killing at 66 days of age. The mean frequency of the large pyroninophilic cells and the plasma cells of those mice was determined in the smear stained with methyl green and pyronin.

RESULTS

BGG antigen was injected into the hind foot pads of the protein deficient mice, 14 days after the onset of the feeding of the protein-free diet, at 45 days of age. Thirty minutes, and 1 hour after the first injection, the contents of fast green histone in the lymphocyte nuclei of the popliteal lymph nodes of the mice were examined (Table 1, Fig. 1). In the control mice fed the regular diet and subjected to the antigen, marked decreases in the content of fast green histone in the small lymphocytes were found 30 minutes and 1 hour after the antigen injection. However, such a decrease in the histone was not found in the protein deficient mice.

Table 1. Fast green histone content in the lymphocyte nuclei of the popliteal lymph nodes of 59-day-old CF# 1 female mice fed a regular diet or a protein-free diet for 14 days and given antigen injection 30 minutes and 1 hour before killing.
(Mean±standard error, n=30)

Groups	30 minutes	1 hour
Regular	100	100
Regular+Antigen	84.5±4.1	79.1±3.2
Protein-free	100	100
Protein-free+Antigen	103.9±5.7	107.5±4.2

Each value for the control or experimental mice which were fed a regular diet or a protein-free diet, without antigen injection are expressed as 100%.

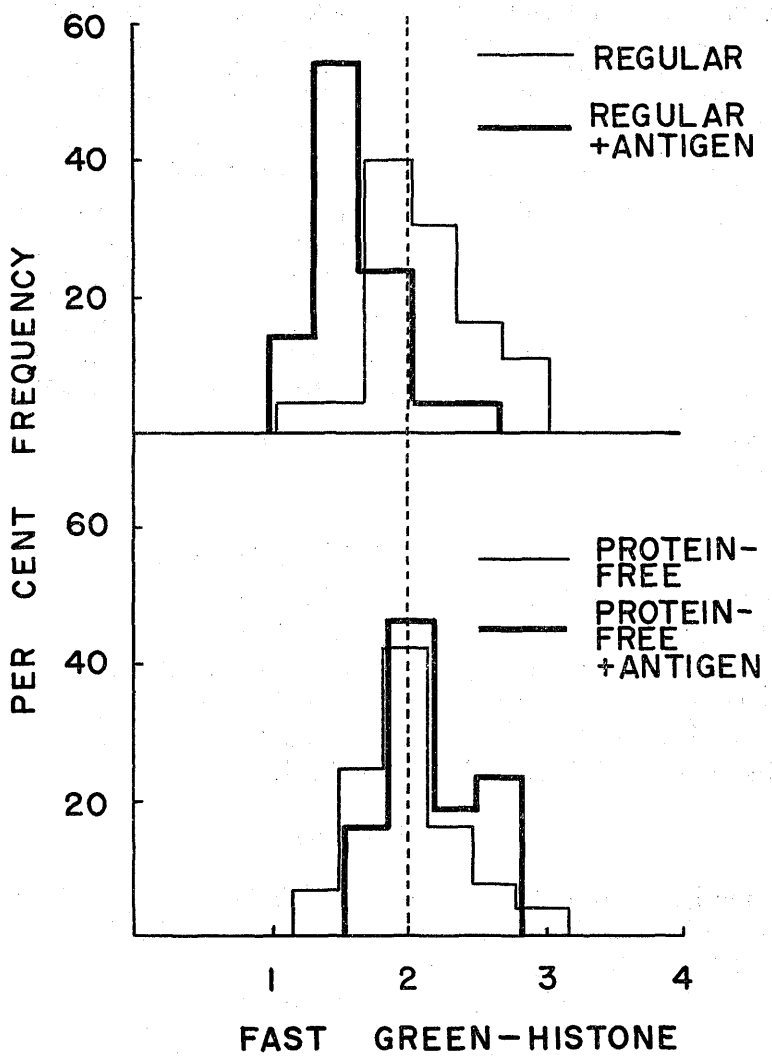

Fig. 1. Histogram of the fast green histone content in the lymphocyte nuclei of the popliteal lymph nodes of 59-day-old CF#1 female mice fed a protein-free diet or a regular diet for 14 days and given antigen injection 30 minutes before killing.

The organ weight and number of nucleated cells, mainly lymphoid cells, in the popliteal lymph nodes per 100 g body weight, were compared between the protein deficient and control mice 3 or 7 days after a typhoid-paratyphoid vaccine injection (Table 2, Fig. 2). The increase in weight and the number of nucleated cells of the lymph node after the antigen injection was seen even in protein deficient mice. However, the

Table 2. Organ weight and number of nucleated cells in the popliteal lymph nodes of female mice fed a regular diet, and those fed a protein-free diet at 45 days of age for 17 or 21 days and given antigen injections 3 or 7 days before killing at 62 or 66 days of age. (Mean±standard error)

Groups	Time after antigen injection (No. of animals)	Organ weight (mg)/100g body weight	No. of nucleated cells ($\times 10^{-6}$)/100g body weight
Regular diet	Control (12)	10.6± 1.1	15.4± 1.5
	3 days (5)	22.9± 3.7	47.1± 6.8
	7 days (6)	50.0±15.4	133.1±40.4
Protein-free diet	Control (8)	4.3± 1.1	8.9± 1.2
	3 days (7)	8.5± 2.0	14.3± 7.5
	7 days (5)	21.6± 6.6	56.5±10.1

Antigen : Typhoid paratyphoid vaccine plus complete Freund's adjuvant (1 : 1, 0.1ml)
Injection site : hind foot pad

degree of increase was not as remarkable as in the control mice.

The frequency of large pyroninophilic cells and plasma cells in the popliteal lymph nodes, 7 days after the BGG antigen injection, are shown in Table 3. Marked increases in the number of large pyroninophilic cells and plasma cells were observed in the popliteal lymph nodes of the control mice. In the protein deficient mice, however, little increase in number of those cells was noticed.

DISCUSSION

Previously, we reported that protein deficiency deeply affected involution of the lymphoid organs, primarily caused by the decrease in the number of lymphoid cells[11]. In the present experiment, it was shown that after antigen stimulation, there were less lymphoid cells (Table 2, Fig. 2), decrease in percent frequency of large pyroninophilic cells and plasma cells (Table 3) in protein deficient mice than in the control mice. These results may be closely related to the lack of a decrease in the content of fast green histone in the lymphocyte nuclei of the protein deficient mice at 30 minutes and 1 hour after antigen stimulation (Fig. 1, Table 1). It has been observed that a transient decrease in the content of fast green histone in normal mouse lymphocytes occurred at 30 minutes after the antigen injection, and then lymphoid cells increased[13)17)]. Some authors have also shown that the depression of antibody titers under protein deficiency could be attributed largely to the reduced number of plaque forming cells (PFC) in the spleen[18),19)]. Malavé et al[20)] suggested that protein deficient animals present a failure in the regula-

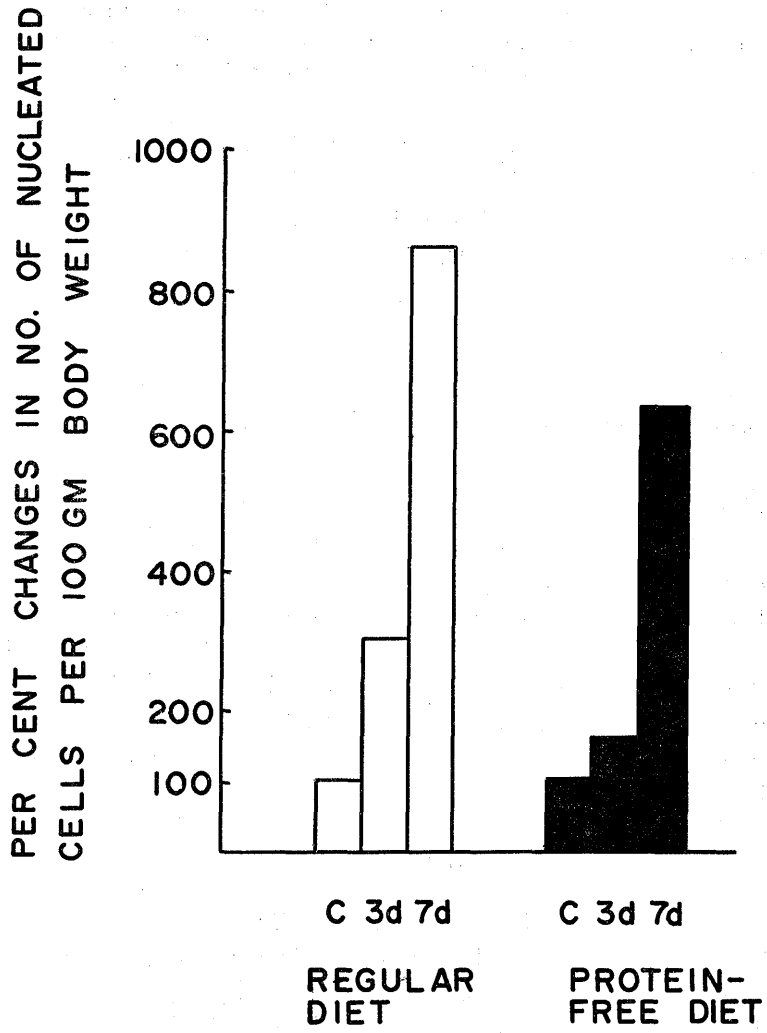

Fig. 2. Percent changes in the number of nucleated cells ($\times 10^{-6}$) in the popliteal lymph nodes per 100 g of body weight, in female mice fed a regular diet and those fed a protein-free diet at 45 days of age for 17 or 21 days and given antigen injection 3 or 7 days before killing at 62 or 66 days of age. Each value for the control mice fed a regular diet, or a protein-free diet without antigen injection are expressed as 100%.
Open column: Regular diet.
Solid column: Protein-free diet.

Table 3. Frequency of large pyroninophylic cells and plasma cells in the smears of the popliteal lymph nodes of male mice fed a regular diet, and those fed a protein-free diet at 45 days of age for 21 days and given antigen injection into hind foot pad 7 days before killing at 66 days of age.
(Mean ± standard error)

Groups (No. of animals)		Large pyroninophylic cells	Plasma cells	Total
Regular	(5)	20.1±4.0	1.9±1.2	25.8±1.9
Regular+Antigen	(10)	56.4±5.8	7.6±1.5	64.0±5.6
Protein-free	(4)	19.2±1.7	1.6±1.0	20.8±2.7
Protein-free+Antigen	(5)	21.3±3.4	2.3±0.5	23.7±3.7

Antigen : Bovine gamma globulin (BGG) plus complete Freund's adjuvant (1 : 1, 0.1ml)
Injection site : hind foot pad

tory mechanism of the IgM response to alloantigens.

It is well known that a marked involution of lymphoid organs, particularly of thymus, is induced by steroid hormones[17)21)]. Furthermore, raised levels of plasma cortisol have been reported in protein-calorie malnutrition[22-24)]. Although we did not measure plasma cortisol of protein deficient mice, intact adrenals and gonads, instead of marked involution of thymus as already reported[11)] indicated that such a decreased immune response might be partly due to a disturbance in lymphocyte function induced by steroid hormone.

SUMMARY

To investigate the effect of portein deficiency on lymphocyte reaction, CF#1 mice were initially fed a protein-free diet (0% casein), or a regular diet (25% casein), and subcutaneously injected with typhoid-paratyphoid vaccine or bovine gamma globulin (BGG) into the hind foot pad.

It was found by microspectrophotometry that the content of fast green histone in the lymphocyte nuclei of the protein deficient mice did not decrease within 30 minutes and 1 hour after the antigen injection, while about a 20% decrease in the control value occurred in mice fed the regular diet.

At 3 and 7 days after the antigen injection, the weight and total number of nucleated cells, and mean frequency of large pyroninophilic lymphoid cells and plasma cells in the popliteal lymph nodes of the protein deficient mice were markedly less than in the control mice.

Therefore, it is concluded that protein deficiency significantly reduces the lymphocyte reaction to antigen stimulation.

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