There is increasing evidence from animal studies that cells of the macrophage-histiocyte series may play an important role in host defence against infection and cancer (8, 25). The state of nutrition is known to be closely related to resistance to infection (24). However, the mechanisms of its relation are still unknown and there are comparatively few reports about the effects of diets on macrophage functions. In general, protein deficiency results in the following dysfunctions of monocytes or macrophages; depressed mobility to inflammatory site (30) and reduction in phagocytic and bactericidal activities of peritoneal macrophages (6) or in carbon clearance (19). In this paper we summarized the cytochemical and functional changes of rat macrophages in various nutritional state.

1) Effects of protein deficiency, pyridoxine deficiency and acute starvation on alveolar macrophages (AM) of rats

Protein malnutrition was achieved by feeding female F344 rats a 5% casein diet for 7 weeks. At 3, 5 and 7 weeks, animals were killed and their AM were obtained by broncho-pulmonary lavage. Functional changes of AM were determined by measuring phagocytosis of latex beads, yeast cells or opsonized sheep red blood cells (SRBC) and the ability to respond to a macrophage-activating factor (MAF) such as lymphokines, which were made by splenic lymphocytes stimulated with concanavalin A bound to sepharose beads (7). After 3 weeks on a 5% casein diet, the number of AM was much lower than in rats on control diet (20% casein), but the abilities of the AM to phagocytose latex and yeast cells were the same as those of control. Our findings are closely related to the data of Price and Bell (21) of decreased number of peritoneal macrophages in protein malnourished mice and consistent with the report of Keusch et al. (11) that peritoneal macrophages from protein-deficient rats showed normal ability to phagocytose latex beads.

Phagocytosis of opsonized SRBC was higher than in control rats but could not be enhanced by in vitro treatment with MAF (14). These data suggested that dietary protein malnutrition affected the number and phagocytic functions of rat AM responsible for host defence in the lung and that in protein malnutrition rat AM could be in a “stimulated” and/or “activated” state. Furthermore, the process of phagocytosis of opsonized SRBC by AM was ultracytochemically studied on two phases as follows; (a) attachment of SRBC to the surface of AM and (b) ingestion of SRBC into the cytoplasm of AM as reported previously (23). In attachment phase, we investigated the ability to form E-rosettes of AM following incubation at 4°C. More than 70% of the AM from rats in the 5% casein group bound more than 11 SRBC per AM whereas only 20% of the AM showed similar binding, and most of AM bound less than 10 SRBC in the 20% casein group.
During initial incubation at 4°C, SRBC were in contact with the surface of almost all AM of the 5% casein group, and some of AM were completely covered with SRBC. The attached SRBC were irregularly shaped or polygonal and in general, they became attached to AM at the edge of deformed regions that were concave or angular. After attachment of SRBC to the tips of microvilli, pseudopodia appeared and extended so that SRBC became surrounded with microvilli of AM. The AM themselves occasionally appeared to be elongated toward SRBC. Transmission electron micrographs showed extension and thickening of the subplasmalemma in the site of attachment of SRBC. The reaction products of membrane ATPase were distributed along the surface of the plasma membrane of AM as reported previously (22). In the 5% casein group these ATPase activity was present on the surface of invaginating areas contiguous to the outer surface of the plasma membrane and also on the inner surface of phagocytic vacuoles in the subplasmalemma in the site of attachment of SRBC. These findings were more prominent in the 5% casein group than in the 20% casein group (15). In regions free of SRBC there were few, if any, vacuoles. Internalization of SRBC was started by increasing the temperature 4 to 37°C. In the process of phagocytosis, SRBC were engulfed into the AM by formation of hemispherical craters and were seen in the phagocytic vacuoles localized in the subplasmalemmal area of the cytoplasm of AM. Extention of pseudopodia due to the action of microfilaments seemed to be involved in engulfment of SRBC as reported by Griffin et al. (9) and Axline et al. (2). A phagocytosed SRBC was surrounded with abundant microfilaments, which were oriented and organized. In the interior of AM of rats fed 5% casein diet the vacuoles fused with preexisting primary lysosomes, which more numerous than in 20% casein group. The lysosomal contents were transferred to the phagocytic vacuoles containing SRBC. In the process of phagocytosis, the stability of the lysosomal membrane may decrease (29) and the induction of lysosomal enzymes depending on synthesis new protein occurs (4). In malnutrition and protein deficiency, intracellular autophagy and the number of secondary lysosomes increase (20). The presence of digestible materials in macrophages leads to a tenfold increase in both the number of lysosomes and the level of lysosomal enzymes (1). Stossel et al. (26) also found higher levels of hydrolytic enzymes in stimulated AM than in unstimulated cells.

In AM that had phagocytosed many SRBC, the number of lysosomes surrounding ingested SRBC was remarkably decreased and the cells tended to be more spherical, with only a few short microvilli.

On the other hand, subplasmalemmal microfilaments have been associated with not only cell movement (5), but also phagocytosis (2). In the 5% casein group, a large number of microfilaments were present in this area and may be necessary for inducing fusion of lysosomes with vacuoles and discharge of their contents as well as for formation of pseudopodia and engulfing of particles. Thus protein deficiency produces an increase in the length of cell process and in the number of phagocytic vacuoles in the subplasmalemmal region and in the number of lysosomes in the interior of AM.

In pyridoxine deficiency, which was achieved by feeding rats a diet without pyridoxine for 4 weeks, AM from pyridoxine-deficient (DEF) and pair-fed control (PF) groups showed a higher degree of phagocytosis of opsonized SRBC than those of rats in ad libitum-fed control (AL) group. After in vitro treatment with MAF
for 4 hr at 37°C, AM from the PF or AL group showed a great enhancement of phagocytic activity than AM from the DEF group, which was slightly enhanced. When the effect of MAF prepared from splenic cells of rats the PF or DEF group on the phagocytosis of AM was observed, MAF from the PF group showed an about 35% increase of phagocytic ability compared to the supernatant of splenic cells cultured with medium only. However, MAF from the DEF group had no effect on phagocytosis of AM. These results suggest that pyridoxine deficiency affects not only phagocytic function of AM responsible for the host defence in the lung but also MAF production from splenic cells (16). Morphological changes of rat AM in pyridoxine deficiency showed the same trend as those of AM in protein deficiency.

Furthermore, of additional interest are the changes of AM functions in acute starved rats. Phagocytic ability to ingest opsonized SRBC by rat AM was dependent upon the term of starvation, showed the change like a wave and the downward trend as a whole. The above-mentioned results suggest that malnutrition such as protein deficiency, pyridoxine deficiency and acute starvation may induce the activation of AM function to a certain extent.

2) Effects of high vitamin A and arginine on macrophage functions during tumor growth and skin tumorigenesis in rats or mice

F344 rats given saline, vitamin A placebo or vitamin A analogues for 4 consecutive days were killed at the following days. Their AM were harvested by lavage with warm saline (37°C). Four days treatment with 100 IU of vitamin A as retinyl palmitate per gram body weight rendered the AM tumoricidal against syngeneic mammary adenocarcinoma cell lines (MADB-100 and -200). AM activated with retinyl palmitate also showed increased ability to phagocytose opsonized SRBC. Other retinoids, such as retinoic acid and retinol, had the same effect of inducing tumoricidal activity in rat AM. AM harvested from normal rats were also rendered tumoricidal by direct interaction with 10⁸ IU ml⁻¹ of retinyl palmitate for 24 hr in vitro (27).

In skin tumorigenesis of mice, high retinyl palmitate diet resulted in significantly lower number and weight of skin tumors induced by 7, 12-dimethylbenz(a)anthracence (DMBA) and 12-o-tetradecanoylpholbol-13-acetate (TPA) compared with those of control group. Numbers of peritoneal lavaged cells and macrophages increased two- to four-fold in mice fed high retinyl palmitate diets. However, there was no significant difference in percentages of peritoneal macrophages per peritoneal lavaged cells. Phagocytosis of opsonized SRBC by peritoneal macrophages increased with increasing contents of retinyl palmitate in diets. High retinyl palmitate diets also increased natural killer cell (NK) activity of mice splenocytes against YAC-1 cells. Cytotoxic activity of mice peritoneal macrophages against allogenic B16 melanoma cells also increased with increasing contents of retinyl palmitate in diets and showed significant enhancements in high retinyl palmitate groups (17, 18). Thus, vitamin A or its analogues at high doses can increase host immune functions able to suppress tumor growth and tumorigenesis.

Next we examined the process of cytolysis of tumor cells by AM from rats treated with vitamin A by electron microscopy and a cytochemical technique to obtain morphological evidence of the enhanced cytotoxicity of the AM. AM from rats given vitamin A had many knobs on their sides and tips. The AM became attached to syngeneic mammary adenocarcinoma cells (MADB-100 or -200) at many focal
points and the tumor cells, then, lost surface microvilli around the contact zones. Detachment of the knobs from the projections on AM was often observed in areas of close associations between AM and tumor cells. The detached knobs were 250 nm in diameter, gave a positive reaction for acid phosphatase, and frequently became attached to the surface of tumor cells. Then, many of the tumor cells in the vicinity of AM exhibited cytolytic changes. From these results, the cytotoxicity of AM stimulated with vitamin A is due to their attachment to the surface of tumor cells and their release of particles with acid phosphatase activity into the narrow space between the cells, and then to uptake of these particles by susceptible tumor cells (12).

The amino acid composition is also important and optimal amino acid composition is required for dietary use in patients. Recently, it has been reported that the addition of excess arginine to basal diet resulted in marked protection against the growth of a transplantable tumor and the tumorigenicity of carcinogens (10, 13). In addition, dietary arginine has been found to have a specific effect on immunologic responses (3). So, we examined the effect of infusion of arginine-rich solution into tumor-bearing rats on the growth of a transplantable tumor (Yoshida sarcoma cells) and the effects of this solution on the nutritional status and immunological responses of the rats. Rats were infused with solutions containing 5.5 and 0.66% arginine for 8 days. Infusions were started at the same time of subcutaneous transplantation of Yoshida sarcoma cells. Arginine-rich solution suppressed tumor growth at the early stage and prevented metastases to the liver and kidney. In addition, arginine supplements enhanced the phagocytic activity of AM. It also resulted in maintenance of a positive nitrogen balance and prevented the increases in the levels of amino acids observed in the control group (28). The suppressive effect of arginine-enriched solution on tumor growth may be due to its activation of the immunologic system, in which the phagocytic activity of AM probably participates.

In conclusion, cellular immune functions such as phagocytic and tumoricidal activities of AM and NK activity could be modulated by dietary manipulation, and the use of an optimal diet maintaining and promoting host immune functions may be useful in protective care of cancer patients.

REFERENCES

7. Fidler, I. J., Darnell, J. H. and Budmen, M. B.: Tumoricidal properties of mouse macrophages
activated with mediators from rat lymphocytes stimulated with concanavalin A. Cancer Res. 36; 3608–3615, 1976.