EFFECTS OF PROTEIN DEPLETION ON NK CELL CYTOTOXICITY AND BONE MARROW CELLULARITY

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Tumor bearing patients have repeatedly been shown to develop states of protein depletion (PD). PD has frequently been associated with depression of acquired immunity. In contrast, little is known about the effects of PD on natural killer (NK) cell cytotoxicity and on bone marrow cellularity and how modulation of these parameters by maleic anhydride divinyl ether copolymer (MVE-2) would be affected.

BALB/c mice 6–10 weeks old were fed normal diets (18% protein) (ND mice) or protein deficient isocaloric diets (PD mice) for 35 days. On days 5, 9, 15, 23, and 35 assays were performed. Three days prior to each assay day, some groups received MVE-2 i.p. In PD mice spontaneous NK activity was reduced by some 80%. After MVE-2 the levels only rose to half the amount of activity of ND mice, although in both groups an increase of NK activity took place. BMC levels of PD mice were also strongly decreased. After MVE-2, increases of BMC in PD mice were quite weak and did not reach the level of normal unstimulated BMC. In PD mice we found body weight loss of 46%, reduction in albumin and total protein of 22% and complete disappearance of prealbumin after 5 days of diets. Preliminary results indicate that repletion with amino acids (NeoAminomel L12.5 o.K.H. Salvia, Boehringer Mannheim Co.) is able restore NK activities and BMC levels.

Key words: Protein deficiency, NK activity, Bone marrow cellularity, Biological response modifiers.

INTRODUCTION

Reduced bioavailability of protein has frequently been associated with impairment of several immune functions. Acquired and specific immune functions in cell mediated and humoral immunity as well as aspecific host defense factors are affected to varying degrees. Children dying of kwashiorkor had an average weight reduction of the thymus gland of approximately 70%. Lymph nodes were small and decreased in numbers and histologic signs of regression in lymphoreticular tissues were a common characteristic. Low numbers of peripheral blood T-cells and depressed proliferative responses to PHA stimulation together with a substantially reduced delayed type hypersensitivity reaction (DTH) to tuberculin have frequently been reported in these disease patterns. Humoral immunity appears to be less affected although the secretory antibody production and reaction is clearly limited, which allows for the binding of bacteria and enterotoxins to membranes and possible facilitates their invasion of the body.

Incomplete phagocytic potential of mononuclear cells and reduced capacity of cell repair and cell regeneration with resulting loss of tissue integrity especially in the mucous membranes of the intestine are frequent consequences of reduced body protein supplies. Natural killer (NK) cell cytotoxicity and bone marrow cellularity (BMC) have received only little attention under the above described nutritional conditions.

In addition, immunomodulation of several cellular elements of the immune system is regulated by biological response modifiers (BRMs). We, therefore, investigated the effects of a selected
BRM, maleic anhydride divinyl ether (MVE-2)\textsuperscript{29,30} on its capacity to regulate NK cytotoxicity and BMC during protein deficiency.

MATERIALS AND METHODS

Animals

BALB/c male mice, 6–10 weeks old, weighing 25 g, were used for experiments. The animals were supplied by the Animal Production Section, Division of Cancer Treatment, National Cancer Institute, Frederick, MD.

Feeds and weight controls of animals

Mice were kept ad libitum on normal feed (18% protein, energy value 4.02 kcal g\textsuperscript{-1}) Diet No. 170593 or on a protein-deficient feed (< 1% protein, energy value 3.97 kcal g\textsuperscript{-1}, Diet No. 170597). Both feeds were purchased from TEKLAD, Madison, MI. The feeds were weighed and allotted in equal amounts to the comparison groups and consumption of both feeds was monitored closely as to make sure that both feeds were eaten in equal amounts. All animals were given water ad libitum. Animals were weighed three times per week on a Ohaus Brainway Electronic Balance to monitor weight behavior.

Drugs

Maleic anhydride divinyl ether copolymer (MVE-2) was kindly supplied by Dr R. Corrano (Adria Laboratories, Columbia, OH). MVE-2 was diluted for in vivo use with PBS [phosphate-buffered saline (Grand Island Biological Company, Grand Island, NY)] and then pH adjusted to 7.0 with 0.1 N NaOH.

Tumor cells

The tissue culture line of YAC-1, a Moloney virus-induced lymphoma of A/Sn origin was used as the target for NK cells.

Assay for splenic natural killer (NK) cell activity

A conventional \textsuperscript{51}Cr-release assay was employed as previously described.\textsuperscript{31} In brief, 1 \times 10\textsuperscript{4} radiolabeled YAC-1 cells [100 \muCi of \textsuperscript{51}Cr (New England Nuclear, specific activity 250–800 mCi mg\textsuperscript{-1}), per 1.0 \times 10\textsuperscript{7} YAC-1 cells at 37°C for 45 min] in 0.1 ml volume were added to graded numbers of splenic effector cells in round-bottomed 96-well microtiter plates. Triplicate cultures were maintained at 37°C and 5% CO\textsubscript{2} for 4 h. At the end of the incubation period the plates were centrifuged for 10 min at 800 g and the supernatants were harvested using a Titertek automatic harvesting system and measured in a \gamma-counter. The percent cytotoxicity was calculated from the formula:

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\% \text{ cytotoxicity} = \frac{\text{experimental cpm} - \text{SR cpm}}{\text{TR cpm} - \text{SR cpm}} \times 100.
\]

SR = spontaneous release as determined by incubation of 1.0 \times 10\textsuperscript{4} tumor cells in 0.2 cc of RPMI 1640 in round-bottomed 96-well microtiter plates for 4 h at 37°C and 5% CO\textsubscript{2}, and TR = total release of radioactivity as determined by adding 0.1 ml of SDS 0.5% to 0.1 ml of 1 \times 10\textsuperscript{4} radiolabeled YAC-1 cells.

Bone marrow cells (BMC)

Single cell suspensions from femoral bone marrow were prepared as described previously.\textsuperscript{32} The total number of viable nucleated cells per femur was counted each for individual mouse in an hemocytometer.

Serum protein determinations

Serum was obtained from whole nonheparinized blood. Quantitative colorimetric determinations were performed for albumin by sigma procedure No. 630\textsuperscript{33} and total protein by Bio Rad protein assay.\textsuperscript{34} Semiquantitative determination of prealbumin was performed by reading levels of prealbumin off a gradient slab gel electrophoresis (5–20% polyacrylamide). The complete procedure is described elsewhere.\textsuperscript{35}

Statistical analysis

The Student's \(t\)-test was used for statistical analysis.

RESULTS

Weight development due to protein-deficient feed (Fig. 1)

Mice on protein-deficient feed (protein deficient diet mice = PD mice) progressively lost weight...