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MURINE LYMPHOCYTE CELL SURFACE PROTEOLYTIC ACTIVITY IS STRAIN RELATED

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SUMMARY: A/J and C57 Br/cdj mice bear the H-2 haplotypes which are generally associated with high responses to bovine gamma globulin and type 3 pneumococcal polysaccharide. A/J has been reported, however, to produce higher levels of antibody than C57 Br/cdj mice against the antigens. The two strains of mice were used as model systems in this study to determine whether the level of lymphoid cell surface proteolytic activity is also genetically controlled. The results of this study illustrate that the level of lymphoid cell surface proteolytic activity is strain related. Since A/J lymphocytes were found to have a significantly higher rate of proteolysis than C57 Br/cdj lymphocytes, a correlation between lymphoid cell surface caseinolytic activity and immune responsiveness is suggested.

Several investigators have provided evidence which suggests a role for proteases in cell proliferation (1-4) and the capacity of lymphocytes to respond immunologically (5). There is, however, little information available concerning the endogenous lymphoid proteases which might function during an immune response. Protease inhibitors were shown to inhibit activation of cells extracellularly (3), and proteolytic activity has been detected on the surface of lymphocytes (6,7). Serine protease inhibitors are effective in suppressing lymphocyte stimulation (2) and the majority of rat and hamster lymphoid cell surface proteases appear to be serine proteases (7,8). However, there is no clear evidence that lymphocyte cell surface proteases do play a role in the immune response.

Immune responsiveness in mice is in part controlled by the H-2 haplotype. C57 Br/cdj mice (H-2^k) produced low levels of antibody to bovine gamma globulin (9) and lower levels of antibody than A/J mice (H-2^a) against type 3 pneumococcal polysaccharide (10). These observations could not be explained by differences in

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haplotype as H-2^k is generally associated with a high immune response against the antigens.

Using A/J and C57 Br/cdj mice as a model system, assays were designed to determine whether the level of lymphocyte cell surface proteases is strain related and could be used to explain the difference in immune responsiveness which has been observed in the two strains of mice.

MATERIALS AND METHODS

A/J and C57 Br/cdj mice (Jackson Laboratories, Bar Harbor, ME) were killed by cervical dislocation. Spleens were removed and cleaned of fat in plastic petri dishes containing phosphate buffered saline or RPMI 1640 (Gibco) prior to teasing with forceps. After filtration through several layers of gauze, the cell suspensions were allowed to stand in petri dishes at room temperature for 20 min for separation of adherent cells, otherwise phagocytic cells were removed by passage over glass wool columns (11). Non-adherent cell suspensions were layered on top of an equal volume of Ficoll-Paque (Pharmacia Fine Chemicals) prior to centrifugation at 400 x 9 (room temperature). The lymphocyte enriched interface was washed three times in the appropriate medium (cold) and cell concentrations were adjusted after counting in a hemocytometer using Turk's solution (0.01% gentian violet in 3% acetic acid) to identify mononuclear cells. Cell viability was determined in trypan blue stain.

Casein nach Hammarsten (E.M. Chemicals, Elmsford, NY) was labeled with [^{3}H]-acetic anhydride by a modified method of Hatcher et al. (12) to a specific activity of 484 cpm/pmol (assuming a molecular weight of 121,700). Caseinolytic activity associated with intact spleen cells was determined using varying concentrations of cells or substrate in a total reaction volume of 120 µl. Assays containing RPMI 1640 were incubated at 37°C in a humid atmosphere of 5% CO_2 to maintain neutral pH. Other assays containing phosphate buffered saline were incubated in a 37°C water bath. The reaction was stopped by addition of 75 µl of cold unlabeled casein followed by 150 µl of 6% TCA (cold). The reaction mixtures were spun in an Eppendorf centrifuge for 5 min and 150 µl of supernatant was counted in 10 ml of Aquasol (New England Nuclear) in a Packard scintillation counter.

RESULTS AND DISCUSSION

Using A/J and C57 Br/cdj mice, unfractionated spleen cells and glass wool column eluants were assayed for caseinolytic activity. There was a higher level of proteolytic activity associated with A/J cells when compared with cells taken from C57 Br/cdj mice (Fig. 1).

Mononuclear cells were removed from spleen cell suspensions by flotation on Ficoll-Paque gradients before assay for caseinolytic activity. There was an increase in proteolytic activity with increased substrate concentration (Fig. 2). Correlation coefficients for A/J and C57 Br/cdj were calculated to be 0.9954 and 0.9916, significant at the $p<.001$ level. The linear regression for A/j was

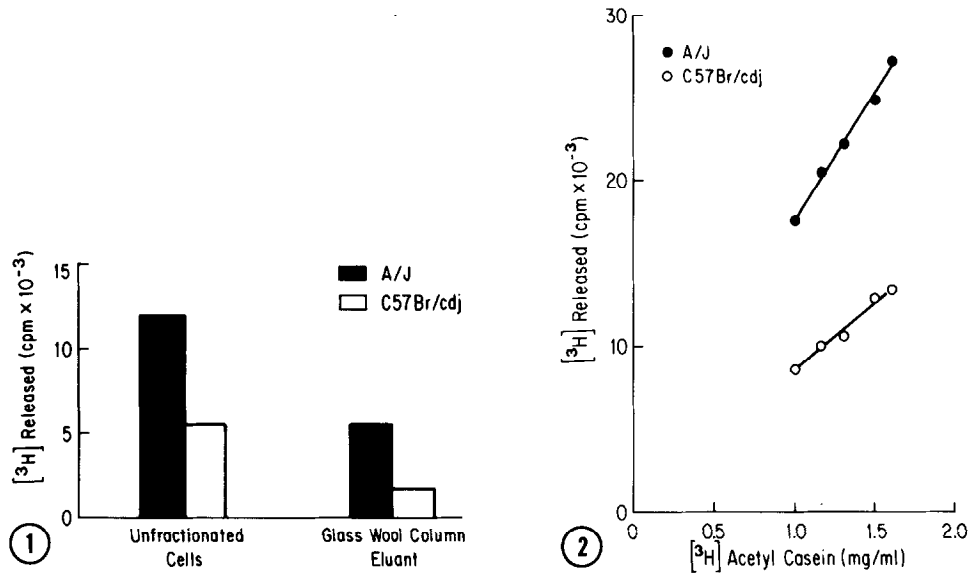

Fig. 1. Protease activities of unfractionated and fractionated spleen cells. Unfractionated spleen cells ($2x10^7$/cc mononuclear cells) and glass wool column eluant cells ($8x10^6$/cc mononuclear cells) were incubated with [^{3}H]acetyl casein (1.7 mg/ml) in a total volume of 120 µl for 2 hours. TCA unprecipitable [^{3}H] was determined.

Fig. 2. Proteolysis of [^{3}H]acetyl casein (varying concentrations) by splenic mononuclear cells. Ten-week-old male mice were sacrificed. Lymphocytes were separated from spleen cells by flotation on a Ficoll-Paque gradient and adjusted to $2.9x10^7$/cc prior to incubation with varying concentrations of [^{3}H]casein in a total volume of 120 µl for 2 hours. TCA unprecipitable [^{3}H] was determined.

calculated to be $y-\bar{y} = 1.5 \times 10^4 (x-\bar{x})$. The linear regression for C57 Br/cdj was calculated to be $y-\bar{y} = 0.82 \times 10^4 (x-\bar{x})$. The slope of A/J is approximately twice the slope of C57 Br/cdj. Covariant analysis was carried out which established that the difference in slopes was highly significant, $p<.001$. There was also an increase in proteolytic activity with increased cell concentration (Fig. 3). Correlation coefficients for A/J and C57 Br/cdj were calculated to be 0.9752 and 0.9903, but was not significant for A/J, $p>.1$, and of borderline significance for C57 Br/cdj, $.1>p>.05$. The lack of frank significance was probably explained by the small number of points. The linear regression for A/J was calculated to be $y-\bar{y} = 7.1 \times 10^3 (x-\bar{x})$. The linear regression for C57 Br/cdj was calculated to be $y-\bar{y} = 3.3 \times 10^3 (x-\bar{x})$. Again, the slope of A/J mice is approximately twice the slope given by C57 Br/cdj mice.

The data obtained from this investigation suggest that the level of murine lymphocyte cell surface proteolytic activity is strain related and that different

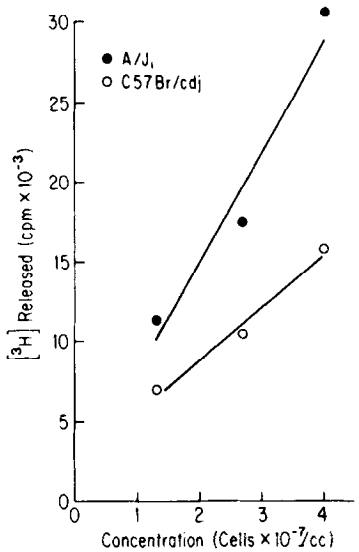

Fig. 3. Proteolysis of [^{3}H]acetyl casein by splenic mononuclear cells (varying concentrations). Ten-week-old male mice were sacrificed. Lymphocytes were separated from spleen cells by flotation on a Ficoll-Paque gradient. A 1.0 mg/ml amount of [^{3}H]casein was incubated for 2 hours with varying concentrations of lymphocytes in a total volume of 120 µl. TCA unprecipitable cpm [^{3}H] was determined.

protease systems function on the surface of A/J and C57 Br/cdj mouse lymphocytes. The finding is consistent with a role for lymphocyte cell surface proteases in the immune response, because A/J mice are higher immune responders than C57 Br/cdj mice, and the rate of caseinolytic activity shown by A/J lymphocytes is approximately twice the value given by C57 Br/cdj mice.

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