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Immune responses in newly developed short-lived SAM mice

I. AGE-ASSOCIATED EARLY DECLINE IN IMMUNE ACTIVITIES OF CULTURED SPLEEN CELLS

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SUMMARY

Using a cell culture system, age-associated changes in immune activities were investigated in newly developed, short-lived mouse strains. These SAM-P strains of mice (H-2^k), which have a remarkably short life span (around 9 months) under conventional breeding conditions, showed an age-associated early decline in several immune functions, as compared to ordinary strains of AKR/J (H-2^k) and C3H/He (H-2^k) mice. Their antibody-forming capacity to T-independent antigen, DNP–Ficoll, and natural killer (NK) cell activity showed a markedly early onset of regression and a sharp decline from the level of control mice at 2 months of age. SAM-P strains of mice have a profound defect in antibody response to a T-dependent (TD) antigen, such as sheep red blood cells (SRBC), thus there was only a feeble antibody response to SRBC as early as the age of 2 months, and a negligible response at a later age. In contrast, the allo-specific cytotoxic T lymphocyte (CTL) response of the mice was as high as that of control mouse strains at 2 months of age and declined little until at least 6 months of age. The early age-related functional decline in the immune system of SAM-P mice suggests that these new inbred strains are appropriate models for investigating the age-related appearance of immune dysfunctions.

INTRODUCTION

New inbred strains of mice termed SAM were developed by Takeda *et al.* (1981). These strains are divided into two groups, SAM-P and SAM-R, the SAM-P strains being markedly short lived. Postnatally they grow normally and are healthy, but then they show early signs of severe loss of physical activity, alopecia, coarse skin, periophthalmic lesions, and increased lordokyphosis of the spine, at around 6 months of age. Cataracts and osteoporosis are also prominent characteristics (Hosokawa *et al.*, 1984a,b; Matsushita *et al.*, 1986). Spontaneous age-associated amyloidosis is an additional outstanding characteristic in one strain, SAM-P/1 (Takeshita *et al.*, 1982), and a unique amyloid fibril protein has been isolated from the livers of this strain (Matsumura *et al.*, 1982; Higuchi *et al.*, 1983; Yonezu *et al.*, 1986; Kunisada *et al.*, 1986; Higuchi *et al.*, 1986).

Abbreviations: CTL, cytotoxic T lymphocyte; DNP, dinitrophenyl; MLC, mixed leucocyte culture; NK, natural killer; PFC, plaque-forming cells; SRBC, sheep red blood cell; TD, T dependent; TI-2, T-independent type-2.

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Evidence has accumulated that the SAM-P strains of mice show a loss of activities at an early age. As the immune system in the mice has not been investigated, we studied the age-associated changes in various immune responses. *In vitro* tests revealed that the antibody-forming ability of SAM-P spleen cells, especially to T-dependent (TD) antigen, is profoundly impaired from early life, yet the allo-specific CTL response is intact.

MATERIALS AND METHODS

Mice

Inbred SAM strains were established from several pairs of AKR/J mice (obtained from the Jackson Laboratory, Bar Harbor, ME) by continuous selection of short-lived litters (Takeda *et al.*, 1981). The SAM strains include several 'senescence-prone' strains (SAM-P) and a few strains without the prominent physiological and pathological characteristics observed in SAM-P strains of mice (SAM-R). Among them, we selected SAM-P/1 and SAM-P/2 (SAM-P mean life span is about 9 months, under conventional breeding conditions, and senile amyloidosis, which starts around at 6 months of age, is one of the most characteristic pathologic findings) and SAM-R/1 (SAM-R mean life span is about 13 months), because these are

the strains most widely studied, from various biological aspects. These strains have been carried longest since establishment as an inbred line. Fifteen general genetic markers and 17 lymphocyte markers were examined in SAM-P, SAM-R, and AKR/J mice. Two genetic markers and two lymphocyte markers differ between SAM-P and AKR/J, three genetic markers and eight lymphocyte markers between SAM-R and AKR/J, and one genetic marker and six lymphocyte markers between SAM-P and SAM-R. Results of serological tests for H-2 antigens indicate that the H-2 regions of SAM-P and SAM-R mice are H-2 K^k, I^k, D^k and H-2 K^s, I^s, D^d, respectively. The genetic differences among the strains suggest that accidental crossing of AKR parents with other strains caused and/or introduced genetic polymorphisms in the SAM strains (T. Takeda, unpublished observations).

AKR/J (H-2^k) mice were originally purchased from the Jackson laboratory. C3H/HeSlc (H-2^k) and BALB/c CrSlc (H-2^d) mice were originally purchased from Shizuoka Laboratory Animal Center (Hamamatsu, Shizuoka). All mice used in the present study were produced in our animal facilities, under conventional breeding conditions. Both sexes were used.

Antigens

Sheep red blood cells (SRBC) were purchased from a domestic dealer and washed three times before use as a TD antigen. Ficoll 400 (Pharmacia, Uppsala, Sweden) was coupled with 2,4-dinitrophenyl (DNP), as described elsewhere (Hosokawa, 1979) to obtain DNP–Ficoll. DNP_{30}–Ficoll, where the subscript represents the number of dinitrophenyl groups per 4×10^5 M_r of Ficoll 400, was used as a T-independent type-2 (TI-2) antigen.

Cell suspensions

Single cell suspensions of spleen cells from each spleen were prepared separately by mincing tissues with scissors and pressing the minced material through a 200-mesh stainless steel screen in cold Eagle's minimum essential medium (Nissui Seiyaku, Tokyo). Cells were washed three times with the medium before use.

In vitro *antibody response*

Spleen cells from each mouse were cultured separately, in duplicate at 5×10^6 cells per well in 24-well tissue culture plates (Corning Glass Works, Corning NY), in 2 ml RPMI-1640 (Nissui Seiyaku) supplemented with 2 mM L-glutamine, 10% fetal calf serum, and 5×10^{-5} M 2-mercaptoethanol. For antibody responses, cells were incubated with 4×10^6 SRBC or 10^{-2} μg/ml DNP-Ficoll in a humidified atmosphere of 5% CO_2 in air at 37°. Cultures were assayed, after 4 days of incubation, by plaque assay.

Mixed leucocyte culture (MLC)

Culture conditions for MLC were the same as for antibody response, except that alloantigens were added. Stimulator cells were prepared from BALB/c spleen and added to cultures at a dose of 5×10^5/well, immediately after 2000 rads irradiation with an X-ray emmitter (Softex-CBM, Softex Co. Ltd, Tokyo). After 5 days of incubation, cultures were harvested and assayed for allo-specific CTL activity.

Haemolytic plaque-forming cell (PFC) assay

Cells secreting antibodies to DNP and to SRBC were enumerated by direct plaque assay in agarose gel on microscope slides, as described elsewhere (Hosono & Muramatsu, 1972). The PFC detected by this method are considered to be IgM antibody-forming cells. In the determination of anti-DNP antibody-forming cells, SRBC trinitrophenylated by the method of Rittenberg & Pratt (1969) were used as indicator cells. PFC assay was done singly for each culture and the mean of duplicate cultures was used as the value of PFC response of each spleen.

Cytotoxicity assay

CTL activity against H-2^d alloantigen and natural killer (NK) activity were determined by ^{51}Cr release assay using, as a target, ^{51}Cr-labelled P815 mastocytoma cells and YAC-1 cells, respectively. Spleen cells, 3×10^5 from 5-day MLC (CTL assay) or 10×10^5 fresh spleen cells from naive mice (NK assay) were mixed with 1×10^4 target cells in 250 μl culture medium in 96-well flat-bottomed microculture plates. The effector to target ratios (E:T ratio), 30:1 for CTL assay and 100:1 for NK assay, were selected because, in preliminary experiments, the E:T ratios gave the highest cytotoxic activity within the range, where E:T ratio and cytotoxic activity showed a nearly linear relationship curve. The effector and target cell mixtures were centrifuged at 300 *g* for a few seconds and incubated at 37° in 5% CO_2. After 4 hr of incubation, 100 μl of supernatant medium from each well was harvested, and radioactivity in the supernatant was determined by a Packard Auto-Gamma 500 gamma counter (Packard Instrument Co., Downers Grove, IL). Percent specific ^{51}Cr release was calculated as follows:

$$\frac{\text{experimental release} - \text{spontaneous release}}{\text{maximum release} - \text{spontaneous release}} \times 100,$$

where spontaneous release is ^{51}Cr release from the well of target cells alone in the culture medium and maximum release is that from the well of target cells alone in 0·1% Triton X-100 (Nakarai Chemicals, Kyoto). Spontaneous release was less than 20% of the maximum release, in every assay. The assay for CTL was carried out in duplicate for each MLC and that for NK activity in triplicate for each naive spleen. The mean of quadruplicate assays from duplicate MLC was used as the value of CTL response for each spleen and that of triplicate NK assays was used as the value of the NK activity.

Statistical analysis

The Student's *t*-test was used to determine the significance of differences between experimental and control groups. Differences were considered to be significant if the *P* value was less than 0·05.

RESULTS

Spleen cell suspensions from individual naive SAM-P, SAM-R, C3H/He, and AKR/J mice at various ages, were tested simultaneously for the following immune activities. In each experiment, as a control, a few C3H/He mice aged 2 months were always included, in addition to other strains of mice of various ages. The C3H/He mice constantly showed a similar level of responses, as indicated in the figures.

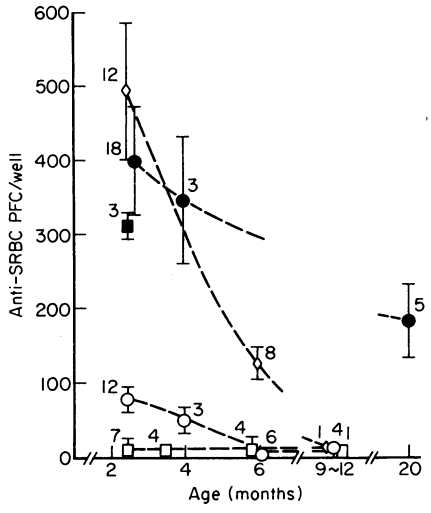

Figure 1. Age-dependent changes in *in vitro* anti-SRBC antibody responsiveness of SAM-P/1 (○), SAM-P/2 (□), SAM-R/1 (◊), C3H/He (●), and AKR/J (■) spleen cells. Data from seven experiments are pooled. Each symbol and vertical bar represents the mean value of several mice and ± 1 SE of the mean, respectively. The bar is omitted when smaller than the symbol. Each figure at the shoulder of each symbol represents the number of mice used.

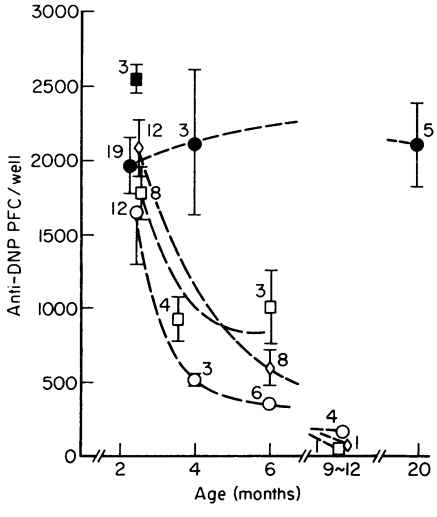

Figure 2. Age-dependent changes in *in vitro* anti-DNP antibody responsiveness against DNP-Ficoll of SAM-P/1 (○), SAM-P/2 (□), SAM-R/1 (◊), C3H/He (●), and AKR/J (■) spleen cells. Data from seven experiments are pooled. See Fig. 1 for details.

Age-associated changes in *in vitro* antibody responses

To T-dependent antigen. As shown in Fig. 1, the anti-SRBC response of cells from the SAM-P strains was feeble, even at the age of 2 months, and was negligible at the age of 6 months. The cells from the SAM-R strain showed as high as a, or even a higher, anti-SRBC response than those from the AKR and C3H/He strains at the age of 2 months, and an appreciable response was still evident at the age of 6 months, although there was a decline in the response from the age 2–6 months.

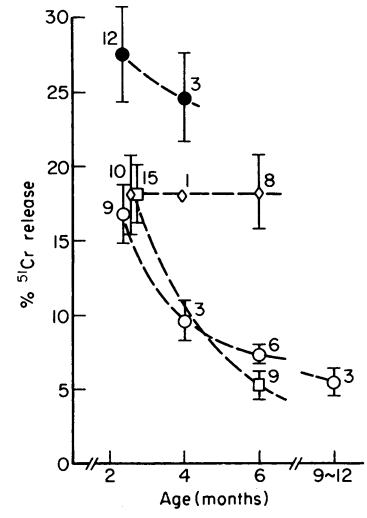

Figure 3. Age-dependent changes in the NK activity of SAM-P/1 (○), SAM-P/2 (□), SAM-R/1 (◊), and C3H/He (●) spleen cells. Data from five experiments are pooled. See Fig. 1 for details.

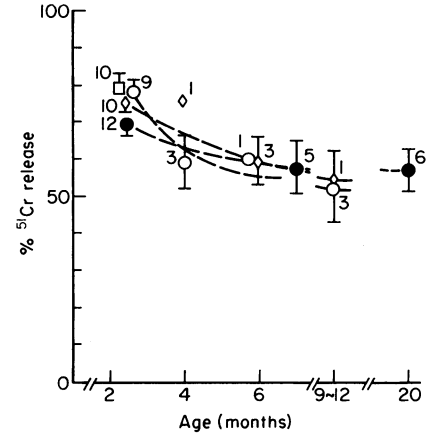

Figure 4. Age-dependent changes in allo-specific CTL-generating ability of SAM-P/1 (○), SAM-P/2 (□), SAM-R/1 (◊), and C3H/He (●) spleen cells. Data from four experiments are pooled. See Fig. 1 for details.

To T-independent antigen. In contrast, the cells from the SAM-P strains at the age of 2 months showed as high an antibody response as those from the SAM-R, C3H/He, and AKR strains, against a typical TL-2 antigen DNP-Ficoll. The antibody response of cells from both SAM-P and SAM-R declined sharply thereafter and an earlier onset of regression than that of C3H/He cells was evident (Fig. 2).

Age-associated changes in NK activity

NK activity against a YAC-1 target of SAM-P spleen cells was as high as that of cells from SAM-R aged 2 months, although it was lower than that of cells from C3H/He. At older ages, a marked difference was observed between these strains. Thus, NK activity of cells from the SAM-P declined with age, while in the SAM-R, NK activity was maintained up to the age of 6 months (Fig. 3).

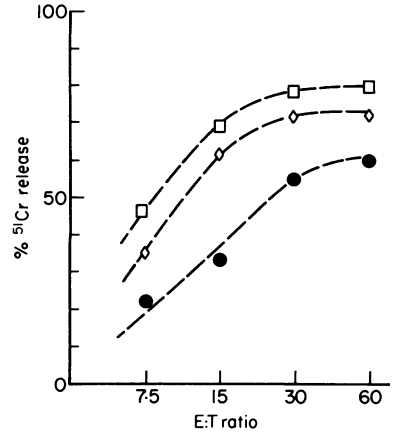

Figure 5. Allo-specific CTL activity of the cells from 5-day MLC of SAM-P/2 (□), SAM-R/1 (◊), and C3H/He (●) spleen cells. Three MLC for three individual mice of each strain were pooled and the CTL were assayed at various E:T ratios indicated on the abscissa.

Age-associated changes in allo-specific CTL response

Cells from both the SAM-P and SAM-R strains showed as high as or even higher allo-specific CTL responses than the spleen cells from C3H/He, at all ages tested. Thus, cells from 2-month-old mice of each strain generated 70–80% CTL activity, and this capacity declined little until at least the age of 6 months (Fig. 4). Comparable CTL activities were consistently observed, at any E:T ratios tested, as shown in Fig. 5.

DISCUSSION

The *in vitro* antibody-forming ability of spleen cells from recently developed inbred SAM strains of mice showed a remarkably early and sharp regression with age. In particular, spleen cells of SAM-P strains of mice, which have the shortest mean life span among the mouse strains used here, had a profound defect in antibody response to TD antigen as early as 2 months of age. In addition, cells from the SAM-P spleen showed an age-associated early and sharp decline in NK activity. Since these characteristics of the immune system of SAM-P (H-2^k) mice were not observed in MHC-identical ordinary strains of mice, the abnormality may not be associated directly with the H-2^k genes. Both sexes of SAM-P mice showed this abnormality in the immune system.

SAM-P mice, when reared under specific pathogen-free conditions, still have a markedly short mean survival time (about 16 months) (Shino *et al.*, 1986), although the mean survival time observed was longer than that of the SAM-P mice reared under conventional conditions. This strongly suggests that the short life span of SAM-P mice is an intrinsic feature. However, specific pathogen-free conditions extend the mean survival time by about 7 months. Therefore, whether the defective antibody-forming activity to TD antigen and age-associated early decline in some immune activities of SAM-P mice have any relationship to the abnormally short life span of these mice is an important question.

The age-associated decline in various biological functions is polymorphic in ordinary strains of mice, with respect to the cellular site of appearance and the age of onset. This is also the case in SAM mice. Senile amyloidosis is one of the most important characteristics of the SAM-P mice used here (Takeshita *et al.*, 1982). Therefore, one might consider that amyloidosis produced the age-related early loss of immune activities in these mice. However, this is not the case since the amyloidosis appears around 6 months of age, much later than the age when the decline of immune activities begins.

It is not clear whether the impaired immune functions of SAM-P mice are inter-related. This issue is now being examined in genetic studies by crossing the SAM-P strains with a normal strain, to observe the segregation pattern of the immune dysfunctions in the F_2 population. The examinations should shed light on relationships among immune dysfunctions senile amyloidosis, and the short life span.

The impaired antibody response to TD antigen of young SAM-P mice is primarily ascribed to T cells rather than B cells, since the antibody response to TI-2 antigen appears intact at that age. This issue is dealt with in the accompaning paper. The observation that spleen cells from SAM-P of various ages proliferate normally to lipopolysaccharide stimulation (T. Takeda, unpublished observations) is further evidence for the intact state of the SAM-P B cells.

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