NOTE

THE LONGEVITY OF ASCITES TUMOR-BEARING MICE
PRETREATED WITH THE HOMOLOGOUS TUMOR EXTRACT

The enhancing effect of pretreatment with tumor preparations on the transplantability of the same kinds of tumors, first studied by Flexner et al. (1907), has been investigated by many research workers. In the effect, as reported by Shear et al. (1954) and Kaliss (1955), may participate certain antigen-antibody system. The correlation between the enhancive phenomenon and histocompatibility-2 locus was clarified immunogenetically by Snell (1955) with the animals of known genotypes. The participation of some antigen-antibody reaction in this phenomenon may be accepted. However, the relation between this enhancive effect and the immune response which may probably occur in the animal body transplanted with a tumor remains still unknown.

The present investigation has been concerned with a search for the proper method for the exact expression of the grade of the enhancement in pretreated animals. The ultimate purpose of these studies is to clarify what relation the antigen-antibody system might possibly have with the enhancive phenomenon. A short report was presented at the 14th General Meeting of the Japanese Cancer Association (Yamada et al., 1955). An ascites tumor (Ehrlich) was used as the tumor material, and the longevity of the ascites tumor-bearing mice was used as a measure of the effect instead of the size of the solid tumors. This method gave more reproducible and less variable results. Originally, the measuring of the survival period of the animals had been introduced by Shear et al. (1951) for the evaluation of the phenomenon; a similar study using the Ehrlich ascites tumor was recently given by Horn (1955). In the present paper the techniques for this test method will be described.

Materials and Methods: Mice: The mouse strains used were dd, dbr, C57BL/6 which had been bred at the Department of Veterinary Diseases of this Institute. The animals were approximately 20 g in body weight. Both sexes were used. The seed materials of the tumor were passaged through dd strain and 2 other strains were used in these experiments. Tumor: the Ehrlich ascites tumor was used after 50 passages or more through the peritoneal cavity of dd mice. Pretreatment: The material of pretreatment was prepared following the procedure as described by Shear et al. (1951); namely, tumor cells were collected by a centrifugation of ascitic fluid about 10 days after intraperitoneal inoculation, ground with sterile sands, added with 3 times the original volume of Hanks'
balanced salt solution at pH 7.0, homogenized by a Waring blender, and then centrifuged at 2,000 rpm for 15 minutes. The procedure of the centrifugation was repeated 4 times using each consecutive supernatant, and the final supernatant was used for the pretreatment. The tumor cell extract thus prepared was injected subcutaneously in 0.2 cc per mouse, in the volume the extract was equivalent to $10^6$ to $10^7$ tumor cells in number. Each group consisted of 10 animals. No detectable tumor was formed at the site of injection throughout the observation period. Animals in the control groups received Hanks’ solution instead of the tumor extract in the same way as the experimental groups. *Inoculation of living tumor cells*: Ascites fluid was taken from the dd mice which had been inoculated intraperitoneally with the tumor cells 7 to 10 days before. On the determination of the cell concentration by a counting method, the ascites fluid was diluted with Hanks’ solution to give several suspensions containing the tumor cells in various number desired. Ten days after pretreatment, each animal was injected intraperitoneally with 0.2 cc of one of the cell suspensions. *Observation*: Following the inoculation with the living tumor cells the animals were recorded daily with regard to deaths and any other changes.

*Longevity of mice bearing the Ehrlich ascites tumor*: The longevity of tumor-bearing animals may be considered as an accumulated expression of the host-tumor relationships which include the growing capacity of the tumor cells and the resistance of the host against the cells. The longevity of mice inoculated with various number of the Ehrlich ascites tumor cells has been investigated using dbr and C57BL/6 strains of mice and it has been found that its distribution pattern may be considered to equal a normal distribution.
As Fig. 1 shows, in case of dbr mice, there was a significant correlation between longevity and inoculum size (correlation coefficient $= -0.988$), if the latter ranged between $10^3$ and $10^8$ cells and was expressed in terms of logarithm. The value of standard error of the mean longevity in each group was various ranging from 0.4 to 0.8 days, never beyond 1 day, if 10 mice were used in a group. All mice developed ascites and succumbed to it, except in some cases where the inoculum size was slightly over $10^3$ cells and some animals survived. Some C57BL/6 mice inoculated with $10^5$ cells showed only subcutaneous tumor mass and survived longer than 30 days. Complete regression of tumor was rather frequently observed in case of $10^4$ cells or less. The standard error of each mean longevity value ranged from 0.6 to 1.0 days with an inoculum size of $10^6$ cells and over 1 day when less than $10^6$ cells were inoculated. Although some correlation was shown to exist between the longevity of the animals and the logarithm of the inoculum size (correlation coefficient $= -0.850$), it was inferior to the case of dbr mice.

As described above, it was found that the standard error of the mean longevity in a group of 10 mice remained less than 1 day, if the inoculum size was greater than $10^3$ cells in dbr mice or greater than $10^6$ cells in C57BL/6 mice.

Changes in longevity of mice pretreated with the fresh extract of Ehrlich ascites tumor: Fig. 2 shows the curves of the cumulative percentage death of the dbr and the C57BL/6 mice, respectively, against the period in days after inoculation with the living tumor cells. In both cases, the inoculum size was varied, consisting of $10^7$, $10^5$ and $10^3$ cells. An experimental group of pretreated mice was compared with a control group in each inoculum size.

In case of dbr mice as seen in the upper chart, the curves of the experimental groups almost always preceded those of the respective control groups. The values of the mean longevity of experimental and control groups are $14.4 \pm 0.7$ and $16.4 \pm 0.5$ days, respectively, with an inoculum size of $10^7$ cells, $17.7 \pm 0.8$ and $19.9 \pm 0.6$ days with $10^5$ cells, and $23.8 \pm 1.3$ and $26.1 \pm 0.8$ days with $10^3$ cells. Summarizing the data, in the pretreated groups the longevity of the mice was shorter and its standard error was larger than in the control groups. When the mean values of the two competitive groups were examined by homogeneity test, there were significant differences in the cases where the inoculum consisted of $10^7$ and $10^5$ cells ($P < 0.05$ in both), but not where $10^3$ cells were used ($P = 0.10$). These experiments were performed several times and it was repeatedly noted that, if the inoculum size was $10^6$ cells or more, the mean longevity of the pretreated group was always shorter than that of the control by 2 or more days. The standard error was less than 1 day in both groups.

In case of C57BL/6 mice as shown in the lower chart, the mean longevity of the pretreated group with an inoculum size of $10^7$ cells was $16.7 \pm 1.3$ days and that of the control group $18.9 \pm 0.8$ days, but the two curves of percentage
death intersected and the null hypothesis in the homogeneity test could not be rejected (0.10 < P < 0.20). The curve of the experimental group with an inoculum size of $10^5$ cells preceded that of the control, but the mortality in the former was lower than in the latter. All 10 mice of the experimental with an inoculum size of $10^3$ cells survived while 4 out of 9 in the control died. Although the difference between the experimental and the control groups were not significant statistically, some of the mice pretreated with the tumor extract died sooner than those which did not receive the extract when a rather large number of living cells was inoculated. On the other hand, when a smaller inoculum size was used, the mortality of the pretreated mice apparently decreased; this fact suggests the occurrence of immunity.

Considering the above-mentioned fact, it can be said that the effect of pre-treatment with tumor material is variable depending upon the mouse strains used, and furthermore, that the longevity and mortality of the tumor-bearing animals may be controlled by different factors independent of one another.
Therefore, the enhancive phenomenon expressed by the shortening of longevity (Shear et al., 1951; Horn, 1955) cannot be considered to be caused by the same mechanism as the one which causes the increase in homoiotransplantability (Kaliss, 1955; Snell, 1955). Since the Ehrlich ascites tumor used by the present authors had originated in a hybrid mouse, its antigenic relationship to the db or the C57BL/6 strain of mice was unknown. Further studies using an ascites tumor from a uniform strain are therefore necessary for the clarification of the biological significance of such an enhancing phenomenon.

**Summary:** The longevity of the Ehrlich ascites tumor-bearing mice was investigated for the determination of the effect of pretreatment with the homologous tumor extract. Using the Ehrlich tumor-dbr mouse system, the enhancement could be expressed by the shortening of the longevity of the pretreated animals. This method appears to be more reliable for analyzing such phenomena.

**REFERENCES**


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