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Effect of Active Immunization of the Rats with Mouse Alphafetoprotein (AFP) Upon Azo-Dye Hepatocarcinogenesis

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SUMMARY

The response to active immunization with mouse AFP of the rats in azo-dye hepatocarcinogenesis was observed. In experiment 1, rats were immunized before receiving azo-dye. And in experiment 2, immunization was started after azo-dye feeding.

The results of experiment 1 were, (1) all of the rats immunized with mouse AFP produced antibodies not only to mouse AFP but also to rat AFP itself, (2) the amount of the antibodies to rat AFP was lower and decreased more rapidly than that to mouse AFP, (3) in the immunized rats, the appearance rate of the so-called primary reaction and the amount of AFP at its peak were strongly suppressed, and the beginning of the secondary reaction was delayed for 4 weeks.

While experiment 2 was resulted in as follows. (1) 100% of the rats produced antibodies only to mouse AFP, (2) wave fluctuations of AFP were characteristic in the immunized rats, and (3) a remarkable difference in the survival rate was recognized between the immunized group and the control group.

Thus, it has been proved that the development of azo-dye hepatocarcinogenesis was significantly suppressed by active immunization, just as it was by passive immunization.

Key words: Active immunization, Alphafetoprotein (AFP), Azo-dye, 3-Me-DAB Hepatocarcinogenesis

INTRODUCTION

We have previously reported that the passive immunization of rats with horse anti-rat AFP antiserum suppresses the development of azo-dye hepatocarcinogene-

sis (Kaneda, H., 1979). And it has been proved that this effect is caused by an immunoreaction between anti-rat AFP antibodies and AFP-producing cells (Suzuki, Y., 1980).

If this is true, a similar effect should also be obtained by active immunization. While, it was reported that the rabbits immunized with heterologous AFP, produced antibodies not only to the heterologous AFP but also to rabbit AFP itself (Nishi, S., 1972).

These reports led us to the present study that an observation of the response to active immunization with mouse AFP of rats in the course of azo-dye hepatocarcinogenesis.

As shown in Fig. 1, two experiments were performed. In experiment 1, rats were immunized before receiving azo-dye, and in experiment 2, immunization was started after having received azo-dye.

We would like to mention about these two experiments, individually.

MATERIALS AND METHODS OF EXPERIMENT 1

32 of 6-week-old male Donryu rats were divided into 3 groups (group A ; 13 rats, group B ; 8 rats, and group C ; 11 rats), and were fed with a normal diet for 12 weeks. During this period the rats of groups A and B were each injected with 50

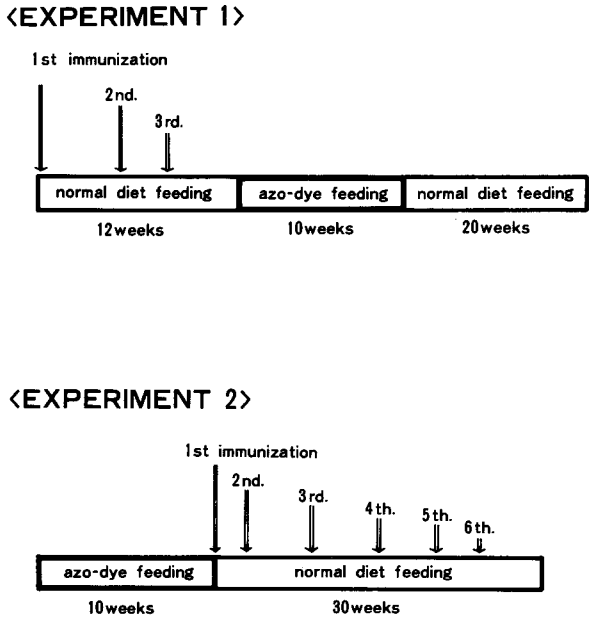

Fig. 1 Scheme of experimental schedule

μg or 100 μg of purified mouse AFP with Freund's complete adjuvant at the week 0, 3 and 8. The animals of group C were injected with only the adjuvant in the same manner, as the control group for immunization. After this period the rats of groups A and C were begun to receive a diet containing 0.06% 3'-methyl-4-dimethylamino-azobenzene (3'-Me-DAB). This feeding was continued for 10 weeks, and then it was returned to a normal diet. While group B was fed with a normal diet continuously, as the control group for hepatocarcinogenesis. The rats of groups A and B were received booster injections with 100 μg of mouse AFP with the adjuvant, at the 10th week of azo-dye feeding.

The amount of the antibodies was measured using a method of reversed rocket immunoelectrophoresis, which was worked out by Taga, one of our co-workers. And serum AFP levels were measured by Mancini's method (See Fig. 2).

RESULTS OF EXPERIMENT 1

12 weeks after the first immunization, all the rats of groups A and B produced antibodies not only to mouse AFP but also to rat AFP itself, as shown in Fig. 3.

Fig. 4 shows the changes of the antibodies in group B. The mean amount of antibodies to mouse AFP was about 1,600 μg/ml at the end of the immunization period, and then it decreased gradually. However, it was re-elevated after a booster injection. Thereafter, once again it decreased gradually. The mean

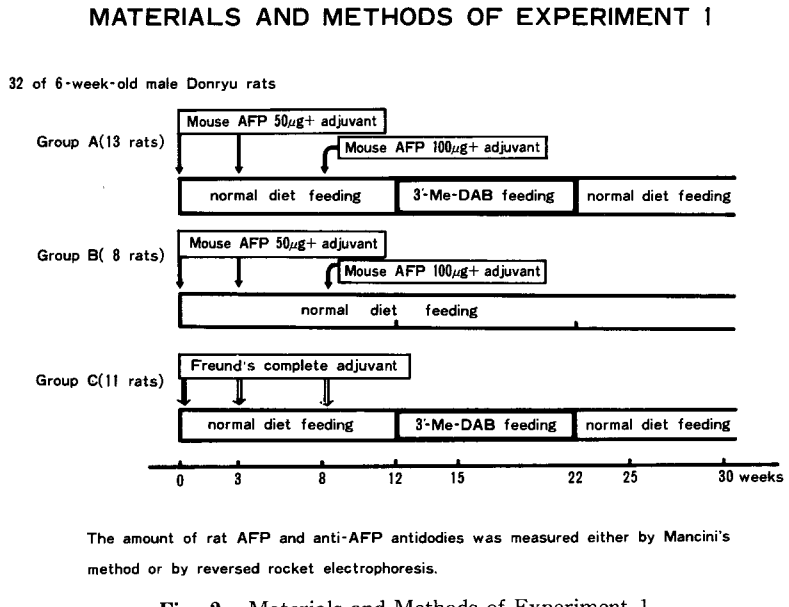

Fig. 2 Materials and Methods of Experiment 1

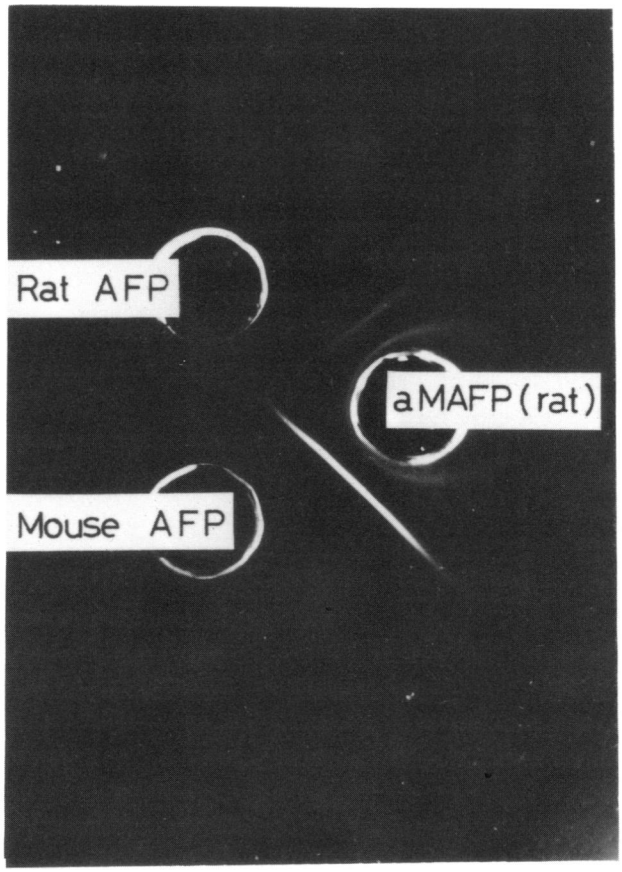

Fig. 3 Antibody to mouse and to rat AFP

amount of antibodies to rat AFP was significantly lower (about 900 μg/ml) than that to mouse AFP at the end of the immunization period, and then decreased rapidly and became undetectable 5 weeks after the end of the immunization period. However, it was also re-elevated after a booster injection. After this once again it decreased rapidly and disappeared 15 weeks after the end of the immunization period.

Otherwise, the changes of the antibodies in group A are shown in Fig. 5. The mean amount of the antibodies to mouse AFP changed similarly to that in group B. While, that to rat AFP decreased more rapidly than that in group B, and became undetectable at the 4th week of azo-dye feeding period. And it was interested that re-elevations after booster injections did not occur in antibodies to rat AFP.

The changes in serum AFP levels after the immunization period are shown in

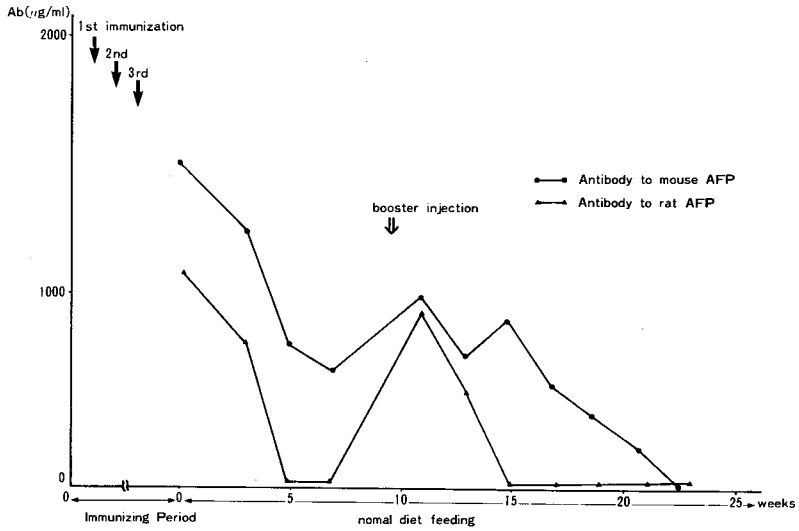

Fig. 4 Change of the mean amount of antibodies to mouse and rat AFP in group B

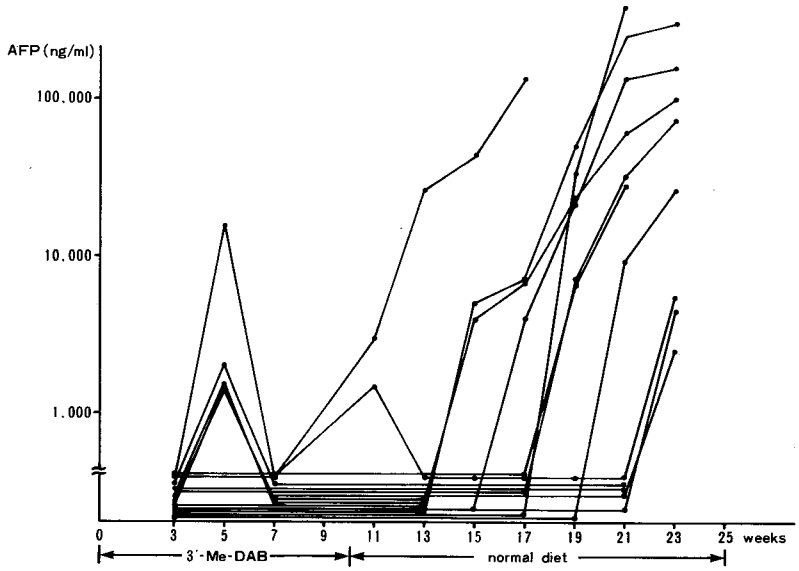

Fig. 5 Change of serum AFP levels in group A

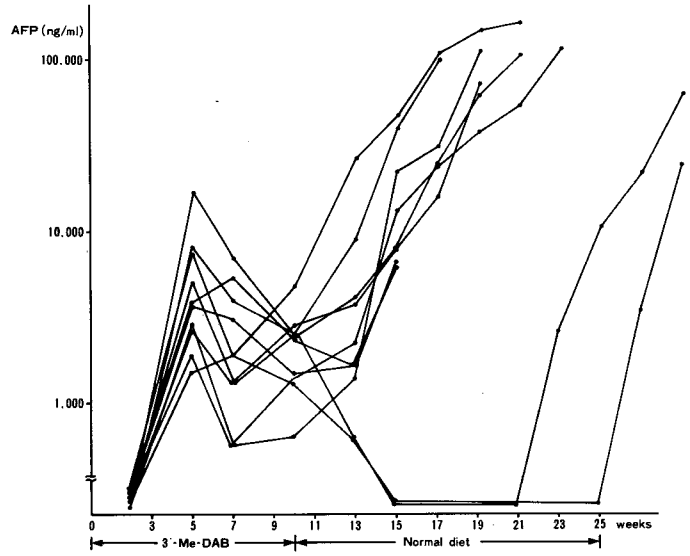

Fig. 6 Change of serum AFP levels in group C

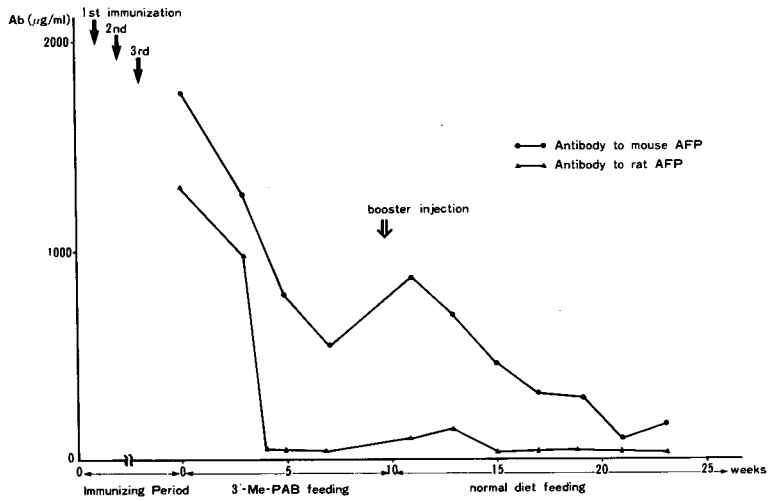

Fig. 7 Change of the mean amount of antibodies to mouse and rat AFP in group A

Fig. 6 and Fig. 7. In group C, the changes were the same as those in an ordinary course of azo-dye hepatocarcinogenesis. Namely, the so-called primary reaction occurred in 80% of the rats and the mean AFP at its peak was about 6,500 ng/m*l*. And the so-called secondary reaction began from the 11th week of azo-dye feeding period (Fig. 6).

On the contrary, in group A, the primary reaction occurred in only 47% and the mean AFP at its peak was significantly lower (2,000ng/m*l*) than that in group C. Also, the begining of the secondary reaction came about 4 weeks later than that in group C (Fig. 7).

DISCUSSION OF EXPERIMENT I

From experiment 1, we can speculate as follows. There is a difference in the strength of affinity to rat AFP between the antibodies to rat AFP and those to mouse AFP, and it is stronger in the antibodies to rat AFP. And so, the antibodies to rat AFP were more rapidly consumed by the rat AFP than those to mouse AFP. Therefore, after the primary reaction the antibodies to rat AFP were no longer exist. And, it is the reason why the secondary reaction could not be completely

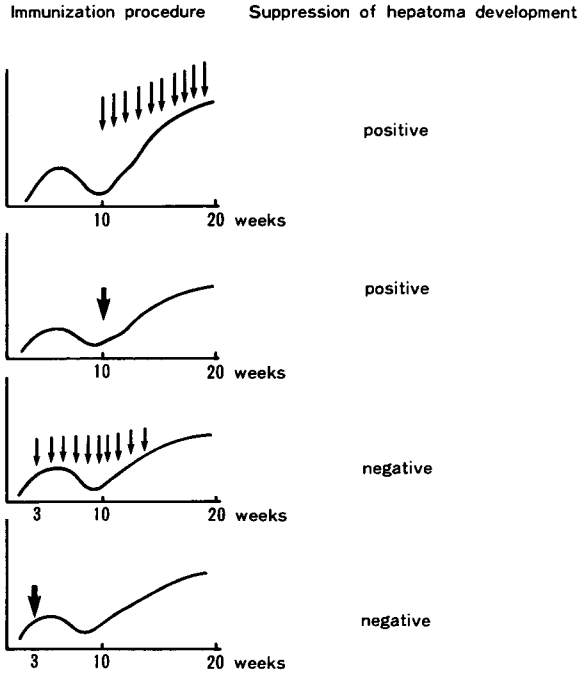

Fig. 8 Abstract of passive immunization experiment

suppressed.

Here, we would like to reconsider the results of the passive immunization. As shown in Fig. 8, a strong suppression of azo-dye induced hepatoma was obtained by serial injections with horse antiserum to rat AFP, when they were begun to give from the 10th week of hepatocarcinogenesis. And the same suppression was obtained by a large single injection (tenfolds as much as dose in one of serial injections), when it was given at the 10th week. But such a suppression was not obtained by serial injections begun to give from the 3rd week. And it was considered that this failure was due to the antibodies against horse serum, which were produced in the rats by repeated injections with horse serum. Actually, antibodies to horse serum were detected by immunoelectrophoresis in the rats at the 12th week of this experiment.

Also, by a large single injection given at the 3rd week, no suppression was obtained.

From these results, we feel it is necessary for sufficient amount of antibodies to exist around the 10th week of azo-dye hepatocarcinogenesis, in order to suppress the hepatoma development.

Considering together the results of the passive immunization and the present experiment, if an immunizing procedure were improved to ensure an adequate amount of antibodies around the 10th week, a more positive effect would be obtained by active immunization.

Thus, experiment 2 was planned.

METERIALS AND METHODS OF EXPERIMENT 2

24 of 6-week-old male Donryu rats were fed with 3'-Me-DAB for 10 weeks. Thereafter, 14 of them were each injected with 200 μg of purified mouse AFP with Freund's complete adjuvant, a total of 6 times (See Fig. 9).

As the control group, the remaining 10 rats were injected with only the adjuvant in the same manner. Measurement of the amount of AFP and the antibodies was performed by the same methods as in experiment 1.

RESULTS OF EXPERIMENT 2

All the rats of immunized group produced antibodies to mouse AFP, however, none of them produced antibodies to rat AFP.

Fig. 10 shows the changes in the mean amount of the antibodies to mouse AFP. The antibodies began to be detected from 5 weeks after the first immunization, and increased up to a maximum of 1,300 μg/m*l* before decreasing gradually.

Differences between the immunized group and the control group were observed in the changes of serum AFP levels and in the survival rates.

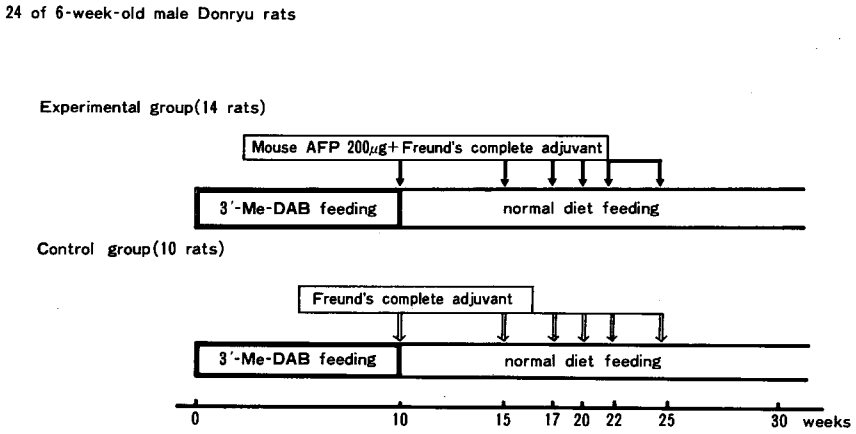

Fig. 9 Materials and methods of experiment 2

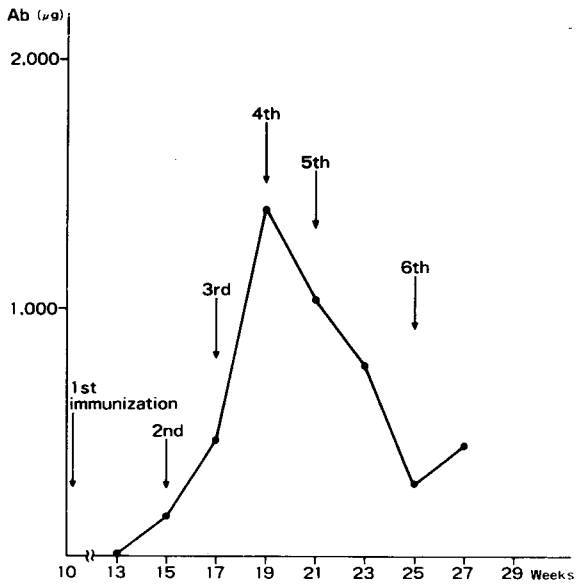

Fig. 10 Anti-mouse AFP antibody titers in rats immunized after 10 week of 3'-Me-DAB feeding (Reversed Rocket Method)

The changes in serum AFP levels are demonstrated in Fig. 11. The secondary reaction began at the 11th week of the hepatocarcinogenesis, the same as the control group. But AFP levels at the begining of the secondary reaction were lower than that in the control group.

And interestingly, wave fluctuations of serum AFP levels were observed in the immunized group.

The survival rate for rats comparing the immunized group and the control group was shown in Fig. 12. All the rats of both groups survived up to the 15th week of the hepatocarcinogenesis. But, at the 23rd week, the survival rate came down to 30% in the control group, on the contrary, 86% of survival rate was maintained in the immunized group. At the 29th week, 65% of the rats in the immunized group still survived, while only 20% of the rats in the control group survived.

DISCUSSION OF EXPERIMENT 2

From experiment 2, three interesting facts were obtained. Namely, the antibodies to rat AFP were never produced in the rats, which were immunized after having received azo-dye, and wave fluctuations of serum AFP levels were observed

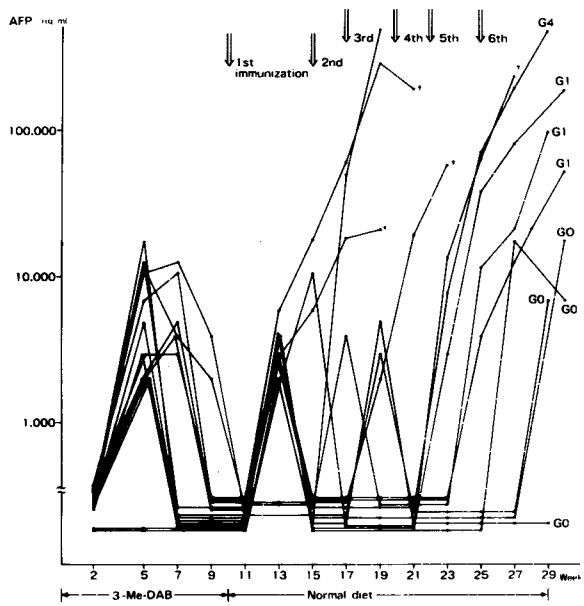

Fig. 11 Serum AFP levels in rats immunized with mouse AFP after 10 weeks of 3'-Me-DAB feeding

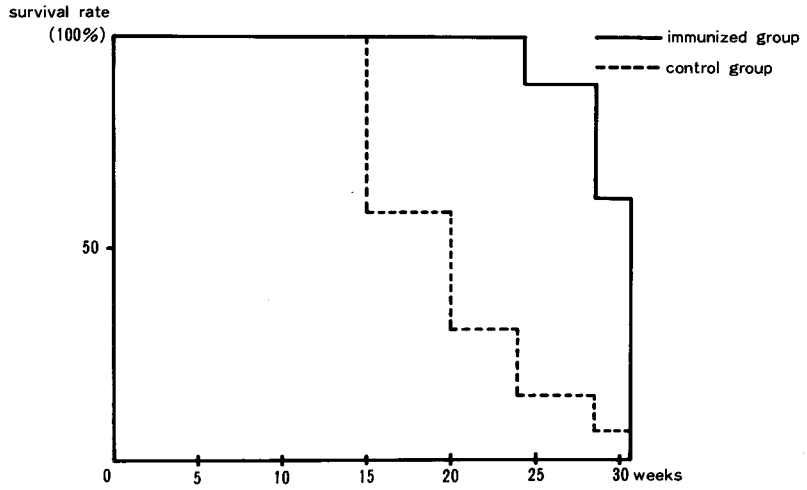

Fig. 12 Comparison of the survival rate between control group and immunized group

in the immunized group, and there was a significant difference in the survival rates between the immunized group and the control group.

When we consider these facts, such fluctuations have never seen either in the control group or in an ordinary course of azo-dye hepatocarcinogenesis, therefore, they are considered to mean the consumptions of serum AFP by the antibodies to AFP. And the facts that there were fluctuations of AFP and a good survival rate in the immunized group under the condition of lacking the antibodies to rat AFP, are considered to mean that not only the antibodies to rat AFP but also those to mouse AFP are effective in suppressing the development of hepatoma. But the facts could also be explained that the antibodies to rat AFP were actually produced, but they were consumed so immediately that they could not be detected in the serum.

TOTAL DISCUSSION

From these two experiments, it has been proved that the development of azo-dye induced hepatoma was significantly suppressed by active immunization, just as it was by passive immunization. But in order to get a more positive effect, improvements of immunizing procedure will be needed. For example, to start the immunization simultaneously to the start of azo-dye feeding, or to give booster injections with rat AFP, may bring better suppressions. And combination of passive immunization with active immunization may also be available.

As for the mechanisms of the effect of active immunization, there are many problems to be solved. For example, are the antibody to rat AFP and that to

mouse AFP, whether different antibodies or only one antibody having crossreactivity? Even if, they are different ones, is only the antibody to rat AFP effective in suppressing the development of hepatoma?

At present, we can not answer to these problems sufficiently, however, we feel that it is reasonable to regard them as different antibodies, because of the differences in the responses to booster injections and in the speed of decrease according to the proceeding of hepatocarcinogenesis.

We hope that the day we can clarify the mechanisms of the effect of active immunization will come in near future.

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