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Growth and Differentiation of Normal and Vaccinia Virus Infected Bone Marrow Stem Cells

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The effect of vaccinia virus (v.v.) has been studied on the growth and differentiation of bone marrow stem cells, transferred between AKR syngenic mice and measured through the number of colonies on the surface of the spleens of host animals. A temporary inhibition of the number of colonies has been found both when the v.v. is administered to the donor animal and when administered to the receptor animal. On the other hand, when the v.v. is administered simultaneously to the donor and to the receptor, there is no interference in the number of colonies.

These results suggest that v.v. interferes, by allogenic mechanism, with the growth and differentiation of bone marrow stem cells, when they are transferred in the above conditions.

It has been shown that vaccinia virus (v.v.) produces growth inhibition of some types of tumor cells (2). On account of interest of this fact it is important to know if the v.v. affects growth of normal somatic cells, by studying the influence of the v.v. on the growth and differentiation of bone marrow stem cells.

In this case the colony forming capacity of stem cells transferred into supralethally irradiated animals, has been used as index of growth and differentiation of these cells. When the stem cells are transferred into supralethally irradiated animals, they grow and differentiate in cellular clones as colonies (1, 6, 7). The number of colonies can be measured by direct counting of nodes which appear on the spleen surfase of host animals (6, 7).

Materials and Methods

Animal. Male, syngenic AKR type mice were used, 8-10 weeks old.

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Irradiation. The mice were irradiated (whole body) at 1,100 R with conventional radiotherapy in the following conditions: 40 cm DFO, a 1 mm copper CHR, and an 0.5 mm aluminium filter dosage rate of 35 rads/min.

Stem cells inoculation. Three hours after irradiation each mouse was injected with 0.1 ml of cell suspension adjusted to 10^6 cells/ml in medium 199 (Biocult)

Spleen extraction and measure of spleen external colonies. Seven days after stem cells inoculation, the mice were killed, and the spleens were removed and put in Bouin's fluid to count the spleen external colonies, which was done as previously described (5).

Virus. The vaccinia virus employed was donated by the Jefatura Provincial de Sanidad (Badajoz), as used for human vaccination. It is a Spanish strain of v.v. attenuated with phenic acid and conserved in glycerine, W.H.O. recognized. The v.v. was administered to the donors, hosts or both donors and hosts AKR mice at times described in results.

Results

The number of colonies formed on spleens of mice that have received bone marrow cells from animals inoculated with v.v. administered two days before the transference is equivalent to that of the controls (without v.v.) (figure 1). The number of colonies is also equivalent to controls when the v.v. has been administered 41 days before trasplant. Nevertheless, no colonies appear when the v.v. has been administered 3 and 16 days before the transference.

The number of colonies on spleens of animals inoculated with v.v. 5 days before receiving cells from normal animals, was zero (table I).

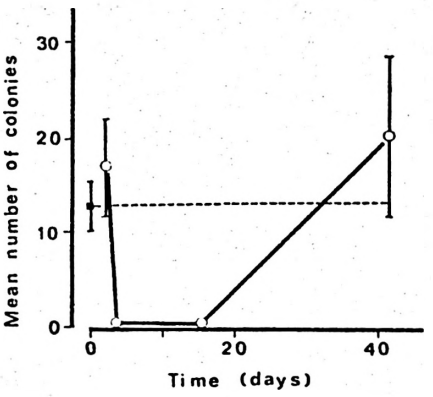

Fig. 1. *Mean Number of Colonies on spleens of receptor animals after bone marrow cells transference from AKR mice inoculated with vaccinia virus* (○) *2, 3, 16, or 41 days before and controls without vaccinia virus* (■). Vertical lines represent the standard deviation from the mean of three experiments with five animals in each one.

Table I. *Spleen colony number (mean±S.D.) on irradiated hosts AKR mice after vaccinia virus (v.v.) inoculation into the hosts or both hosts and donors AKR mice.*

Donor animals	Hosts animals	n	Colonies
without v.v.	without v.v.	5	12.2±1.92
without v.v.	with v.v.**	5	0
with v.v.*	with v.v.**	8	14±4.87

n = Number of experiments (each experiment has three animals).
* = The injection of v.v. was done 5 days before the donor was killed.
** = The injection of v.v. was done 5 days before receiving the stem cell.

Table II. *Haematocrit and leucocite number (mean±S.D.) in AKH mice after different times of vaccinia virus (v.v.) inoculation.*

Days since v.v. inoculation	n	Haematocrit (%)	Leucocite (number/mm³)
0 (control)	5	47.6±2.06	4,800±1,575
10	7	44.6±2.71	6,300±1,314
70	7	44.9±3.18	5,875±1,281

n = Number of animals.

The number of colonies on the spleens when the donor and receptor have received the v.v. 5 days before, is equivalent to the number of colonies in the control groups (donor and receptor without v.v.) (table I).

The haematocrit and leucocite number in peripheric blood stays constant 10 and 70 days after the v.v. inoculation of animals (table II).

Discussion

The stem cells from animals previously inoculated with v.v. lose the capacity to form colonies, and this fact shows that v.v. interferes in some way with the growth and differentiation of the transferred cells.

These growth and differentiation interferences could be explained in two ways: *a*) the v.v. damages the intrinsic mechanisms of cellular growth, and *b*) the v.v. introduces modifications in the membrane proteins of the stem cells giving rise to a growth inhibition which seems to be allogenic.

The first hypothesis does not seem probable because the number of white blood cells and haematocrit (table II) does not diminish in mice inoculated with v.v. 10 and 70 days before killing, whereas the high turnover of these cells is maintained, which could mean that stem cells have not been strongly damaged.

The second hypothesis seems to be more probable. It has been shown that v.v. induces modifications in the histocompatibility antigens of cell membrane (3). As histocompatibility differences between donant and host reduce the number of colonies (5) and the stem cells do not have immunological activity and the host has been supralethally irradiated losing its immunological activity as well (4), the observed growth inhibition may be produced by an allogenic mechanism.

If the v.v., injected into the donor animal, was responsible for the induction of these differences, the same would occur when the v.v. was injected into the receptor. In fact, when the v.v. is injected into the receptor animals and not into the donors, there are no colonies (table I). This supports the hypothesis that the v.v. induces the same antigenic changes on the receptor animals, as occurred with the donors in the same way.

If the above hypothesis is true, the injection of v.v. into both donors and receptors should produce identical changes in both affecting the number of colonies. The results of this experiment (table I) show that, in this case, the number of colonies is normal. These results suggest that v.v. induces specific changes in the cell membrane proteins, which are responsible for an allogenic growth inhibition of bone marrow stem cells.

Resumen

Se ha estudiado el efecto del virus vacuna sobre el crecimiento y diferenciación de las células madre de medula ósea trasplantadas entre ratones singénicos AKR, utilizando como parámetro de medida el número de colonias sobre la superficie de los bazos de los animales huéspedes. Se ha encontrado una inhibición temporal del númetro de colonias cuando dicho virus ha sido administrado al animal donante o al animal receptor. Cuando el virus vacuna fue administrado simultáneamente al donante y receptor no hubo interferencias en el número de colonias.

Estos resultados sugieren que el virus vacuna interfiere, probablemente por un mecanismo alogénico, con el crecimiento y diferenciación de las células madre de medula ósea, cuando dichas células son trasplantadas en las condiciones mencionadas.

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