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EFFECTS OF RADIATION DAMAGE TO BONE MARROW ON SUSCEPTIBILITY OF CHICKS TO ERYTHROLEUKEMIA VIRUS

by

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Studies of fowl and murine virus-induced leukemias indicate that several host factors are responsible for the susceptibility. Increasing immunologic maturity of the host and increasing cellular differentiation, together with decreasing proliferative activity of the target cells, are associated with decreasing susceptibility to virus action in some systems (LAGERLÖF & SUNDELIN 1963). In murine leukemia systems, the response of the animal to the virus can be markedly enhanced by roentgen irradiation or by urethane, which cause damage and subsequent regeneration of the thymus, the target organ of the virus (KAPLAN 1967, HARAN-GHERA & KAPLAN 1964).

The present investigation was undertaken with a view to determine whether the pathogenic effect of the fowl erythroleukemia virus can be altered by modifying the cellularity and the degree of cellular differentiation of the target, i.e. the bone marrow. Bone marrow changes were produced by exposing the chicks to whole body irradiation. The sequential changes in the bone marrow, after irradiation, were followed in a pilot study. Based on this experiment, a study was

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performed on the pathogenic effect of the erythroleukemia virus inoculated in the chicks, in the successive phases of post-irradiation damage and regeneration of the bone marrow.

Material. Random-bred white Leghorn *chicks* (Vanhammar, Vansbro) were used throughout. They were kept in artificially heated cages with free access to commercial chick feed and water; irradiated and non-irradiated chicks were kept in separate cages but otherwise the conditions were the same.

The properties of the erythroleukemia *virus* and the disease evoked by it have been described in detail earlier (LAGERLÖF 1960). The source of the virus was a lyophylized pool of medium from cultures of erythroleukemic bone marrow, diluted to the original volume with distilled water. The dose was 0.2 ml intravenously.

Irradiation. The chicks were exposed to a single dose of 600 rad unfiltered ^{60}Co, the exposure time being 19 minutes and the source-target distance 150 cm. The irradiation resulted in about 10 per cent mortality within the first two days. The preliminary pilot studies had indicated that this dose caused considerable damage to the marrow in spite of the low mortality rate.

For *bone marrow biopsy,* the right femur was exposed under general anesthesia, and a hole of 2 mm diameter was drilled through the cortex of the bone into the medullary cavity. A piece of bone marrow, about 5 mm in length, was aspirated through a needle of 1 mm diameter. The hole was covered with muscular tissue and the operation area closed with silk.

Histologic examination. Bouin's fixative was used for the biopsy material of bone marrow and other tissues collected for histologic examination. Ordinary paraffin sections were prepared and stained with hematoxylin-eosin.

All histologic sections were coded and the examination was made without knowledge of the time of the irradiation treatment. The control marrows were also included, as coded histologic sections, in the experimental material.

Necrosis and edema, indicative of recent damage, were registered, as well as increased fat infiltration with decreased cellularity and signs of erythroid or myeloid regeneration. All these findings were semi-quantitatively registered according to a 4+ scale, where 0 denoted normal findings, 1 or 2+ slight to moderate changes, and 3 or 4+ severe changes.

The obtained biopsy material was sometimes too small to permit a reliable histologic examination. These specimens were excluded when calculating the virus activity figures in the various groups in Table 2.

Calculation of virus activity. The number of chicks dying from leukemia, versus number of virus-inoculated chicks corrected for intercurrent deaths, have

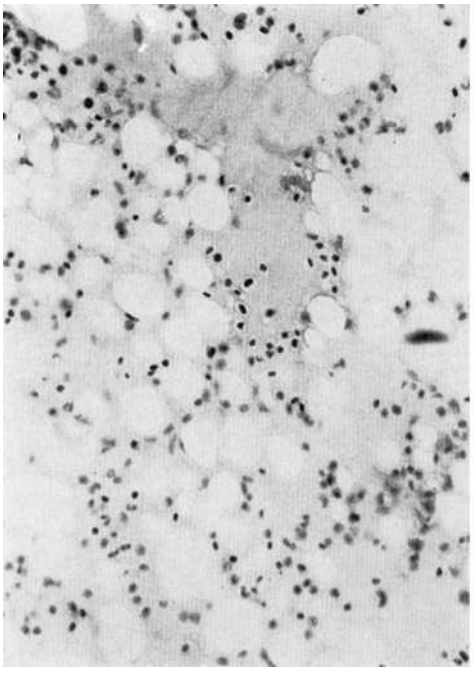

Fig. 1. Acute irradiation damage of bone marrow at 4 days. Edema and few viable erythrocytes (centre) in the necrotic marrow. Hematoxylin-eosin. ×250.

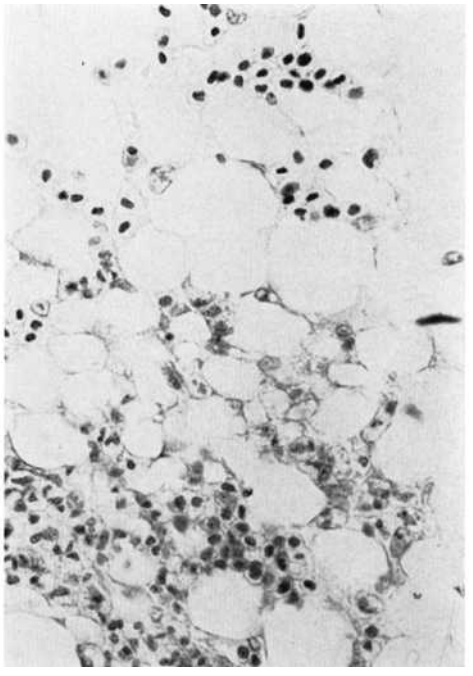

Fig. 2. Acute irradiation damage with edema and cellular necrosis 4 days after irradiation. Persisting erythropoietic activity in some sinusoids (bottom). Hematoxylin-eosin. ×350.

been recorded in tables. In addition, the susceptibility to the virus has been calculated according to

$$\sum \frac{1}{t} \Big/ N$$

where t is the latent period from virus inoculation to death and N is the corrected number of virus-inoculated chicks.

Experimental groups. Chicks were exposed to a single dose of 600 rad in one experiment (1) intended for the evaluation of radiation damage to the bone marrow. The chicks were killed by decapitation 4, 8, 12, and 20 days after irradiation, and all marrow in both femora and tibiae was collected separately for histologic examination. Four to six untreated normal chicks of corresponding ages furnished the control material.

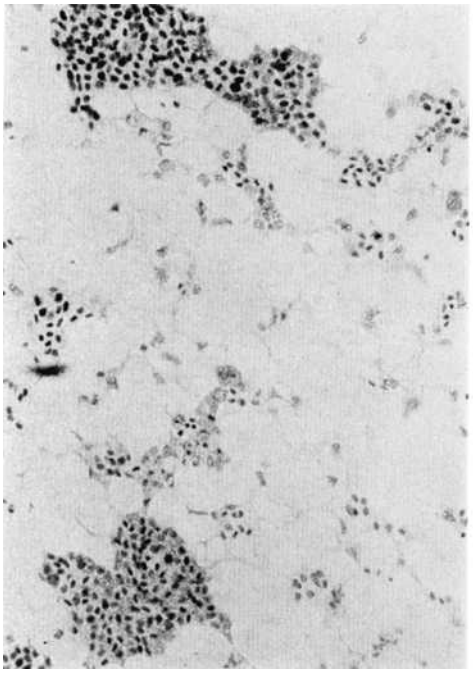

Fig. 3. Hypoplastic bone marrow with increased fat content 8 days after irradiation. Slight myeloid activity with two sinusoids presenting moderate erythropoietic activity. Hematoxylin-eosin. ×250.

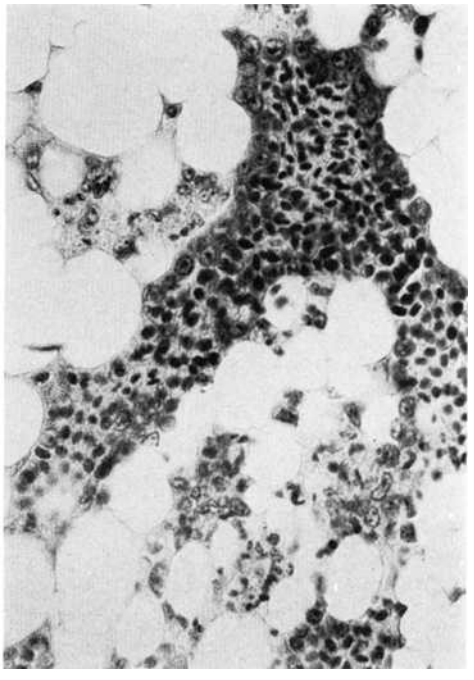

Fig. 4. Sinusoid with moderate erythropoietic activity 12 days after irradiation. Slight myeloid activity in the extrasinusoidal tissue is also evident. Hematoxylin-eosin. ×350.

Groups of twelve 1-day-old chicks were in another experiment (2) exposed to 600 rad and bone marrow biopsy was performed 2, 8 and 20 days after irradiation. Following biopsy, the chicks were inoculated with erythroleukemia virus. Histologic examination of the bone marrow, spleen and liver was performed in all the chicks that died of leukemia or were killed at the termination of the experiment, 60 days after inoculation. Corresponding groups of non-irradiated chicks constituted the control material. The control groups were virus-inoculated at the same time as the experimental groups.

All the groups were kept for 2 months and then the remaining chicks were killed and examined.

Intercurrent deaths. Five out of a total of 72 chicks, in the experimental and control groups, died intercurrently before leukemia had developed. These five chicks were excluded from any further calculations.

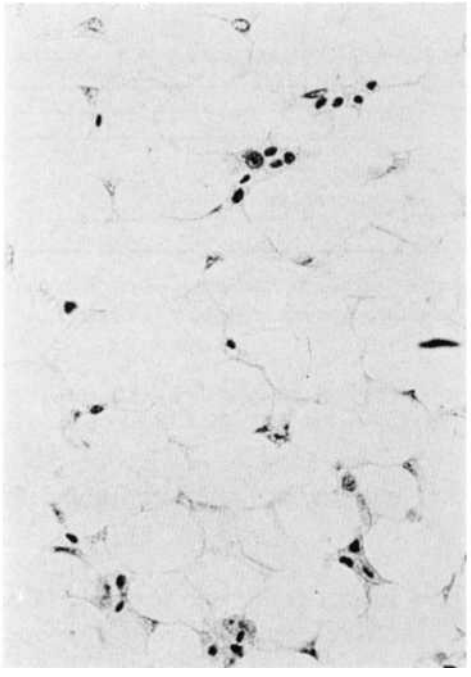

Fig. 5. Aplastic bone marrow 20 days after irradiation. Hematoxylin-eosin. ×350.

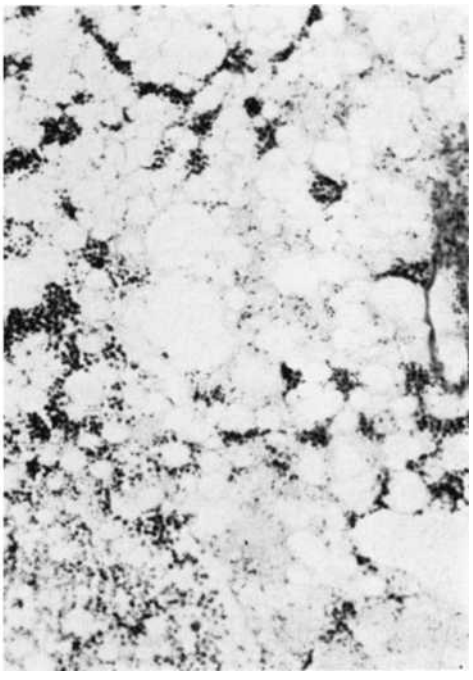

Fig. 6. Low-power view. Persistent edema and patchy necrosis of bone marrow 20 days after irradiation. Hematoxylin-eosin. ×100.

Results

Evaluation of radiation damage to bone marrow

Four days after irradiation. The changes at microscopy of the bone marrow 4 days after irradiation with 600 rad were uniform in type, although the degree differed. All samples exhibited moderate to marked necrosis, haemorrhage, and edema associated with hypocellularity and marked depression of the hematopoietic activity (Fig. 1). Slight erythropoietic activity was evident in some areas (Fig. 2).

Eight days after irradiation. Necrosis of the bone marrow was still a common finding 8 days after irradiation. Haemorrhage and edema were present to about the same degree as in the 4-day group. The changes were generally associated with increased fat infiltration in the marrow, and more or less complete aplasia in large areas. Usually, only the peripheral parts of the marrow presented hematopoietic activity (Fig. 3). The marrow from one of the four chicks exhibited

Table 1

Erythroleukemia virus activity in irradiated and non-irradiated chicks

Days between irradiation and virus inoculation	Radiation plus virus		Virus only	
	Number of dead versus number of inoculated	$\sum \frac{1}{t} \Big/ N$	Number of dead versus number of inoculated	$\sum \frac{1}{t} \Big/ N$
2	7/10	0.066	7/10	0.070
8	8/12	0.057	8/11	0.071
20	7/12	0.047	7/12	0.056
	22/34	0.056	22/33	0.065

slight granulopoietic activity and the other samples had mild erythropoietic activity.

Twelve days after irradiation. The marrow in this group was regularly fatty and hypocellular, and necrosis was only slight compared with earlier groups. Hematopoietic activity was limited to the periphery where most samples had slight to moderate erythropoietic activity (Fig. 4). One of the four birds also exhibited moderate granulopoietic activity.

Twenty days after irradiation. The marrow in this group was usually hyperplastic, with moderate to marked erythropoietic and granulopoietic activity. The fat content of the marrow compared with that of the abovementioned groups and the controls was reduced. One of the four chicks had changes that deviated considerably from the others. This chick had a hypoplastic fatty marrow with evidence of slight erythroid and myeloid cell proliferation (Fig. 5). No edema or cellular necrosis were seen in the marrow.

The bone marrow damage and regeneration were uniformly distributed. The marrow of both femora and tibiae was essentially similar in each chick. A biopsy sample of the femur marrow was considered to represent the radiation damage to the whole marrow in the following experiments on the combined effects of irradiation and erythroleukemia virus.

Combined treatment of chicks with radiation and erythroleukemia virus. The combined treatment with radiation and erythroleukemia virus in the control chicks gave overall results similar to those in which treatment was with virus alone (Table 1).

Table 2

Correlation of erythroleukemia virus activity with the degree of radiation marrow damage (A) and evidence of erythroid regeneration (B)

	Number of dead versus number of inoculated	$\sum \frac{1}{t} \Big/ N$
A		
Irradiated chicks with advanced marrow damage (3 and 4+)	4/13	0.025
Irradiated chicks with slight marrow damage (1 and 2+)	17/21	0.092
B		
Irradiated chicks with histologic evidence of erythroid regeneration	17/25	0.062
Irradiated chicks without histologic evidence of erythroid regeneration	4/9	0.028
Non-irradiated control chicks	22/33	0.063

The incidence of leukemia at corresponding ages was almost identical in the experimental groups and the control groups. The susceptibility of the chicks as calculated according to

$$\sum \frac{1}{t} \Big/ N$$

was identical in the experimental groups and the control groups at 2 days and 20 days after irradiation. At 8 days, the control group had a slightly higher susceptibility.

The biopsies revealed that these experimental groups differed from the above-mentioned groups by having greater intra-group variations of radiation damage. Thus, some of the chicks that were inoculated with virus 20 days after irradiation had persistent widespread cellular necrosis of the marrow or aplasia with marked fatty infiltration (Fig. 6). Placing the birds according to the presence of severe marrow damage and advanced hypocellularity into one group (3 and 4+) and those according the presence of slight to moderate necrosis or hypocellularity into another (1 and 2+), irrespective of the time of virus-inoculation, gave the figures recorded in Table 2.

The chicks with severe marrow damage histologically classified as 3 or 4+ had markedly reduced susceptibility for the virus and only four out of thirteen chicks developed leukemia. Of the twenty-one chicks with slight or moderate marrow damage seventeen developed leukemia; this is an incidence higher than

in the control chicks. The lower susceptibility correlated with severe marrow damage is also evident from the calculated activity values according to

$$\sum \frac{1}{t} \Big/ N$$

where both the latent periods and incidence influence the values. It is also obvious from Table 2 that erythroid regeneration in the radiation-damaged marrow contributes to the susceptibility of the chicks to the virus. Chicks with evidence of erythroid regeneration in the marrow were much more susceptible than chicks without evidence of erythroid regeneration.

Histologic examination of the irradiated and virus-inoculated chicks, dying or killed in the terminal stage of erythroleukemia, revealed persistent bone marrow necrosis in four chicks, with only slight leukemic infiltration despite heavy leukemic infiltration in other organs (Figs 7 and 8).

Discussion

The results obtained clearly demonstrate that advanced cellular necrosis of the bone marrow produced by a single dose of irradiation inhibits the development of erythroleukemia (Table 2). The most likely cause of this inhibitory effect is that the target cells for the virus are damaged and reduced in number. The marrows with 'advanced necrosis' contained very few viable cells scattered throughout its highly edematous marrow. There was a striking difference in susceptibility to the virus between this group and the one with only slight or moderate damage. The latter group had an increased susceptibility also in comparison with unirradiated control groups. This is interpreted as being due to rapid regeneration whereby the marrow will contain actively proliferating erythroid cells which are the target of the virus. The difference in susceptibility to the virus might thus be explained by the shortage or abundance of immature, proliferating erythroid cells. This hypothesis was checked by grouping the experimental chicks according to histologically demonstrable erythroid proliferation in one group and absence of erythropoietic activity in another group (Table 2, group B). The erythropoietically active group was more susceptible to the virus than the inactive group. The activity values were not as high as in the group classified as having 'slight radiation damage'. The difference is small, however, and largely due to longer latent periods in the group classified as presenting evidence of erythroid regeneration.

The total leukemia incidence is almost identical in the irradiated and the non-irradiated groups, and only shorter latent periods increase the activity values in

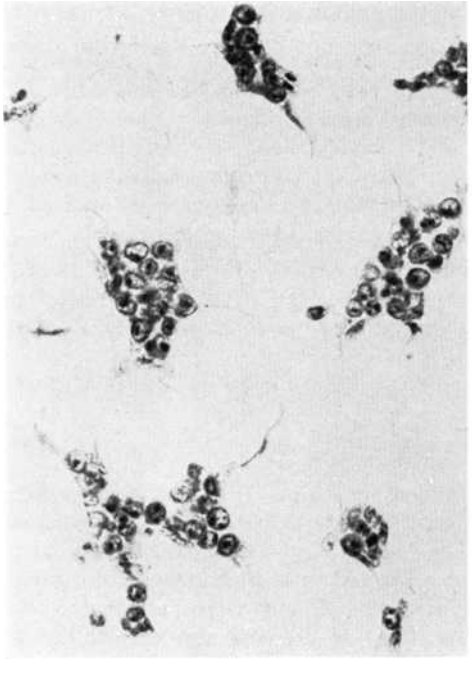

Fig. 7. Fat marrow containing scattered foci of leukemia cells in a chick dying of fulminant erythroleukemia following exposure to radiation 17 days previously. Hematoxylin-eosin. ×350.

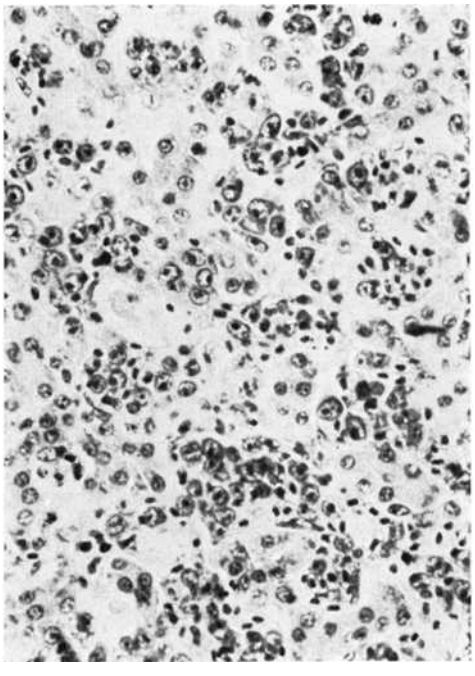

Fig. 8. Liver from the same case as in fig. 7. Heavy leukemic infiltration with sinusoids distended by leukemia cells. Hematoxylin-eosin. ×350.

the unirradiated group. It is thus obvious that the decrease in susceptibility dependent upon radiation damage is compensated for by the increased susceptibility of the chicks with only slight marrow damage.

The dose of virus administered has been the same in all the groups. The short latent periods from inoculation to death indicate that the virus preparations have been very active. No further attempts have been made in the present study to determine whether changing the virus dose would influence the response of the irradiated marrow.

The results obtained support the hypothesis that the pathogenic effect of this oncogenic virus is primarily dependent upon the presence of specific target cells. Whether the increased susceptibility reflects only the increased number of actively proliferating erythroid cells, or whether irradiation has also caused a qualitative change in the cells cannot be settled by the results so far obtained. It is interesting to note that at the terminal stage of the leukemia only slight

leukemic infiltration was present in the fatty or edematous marrow of some of the irradiated chicks, despite heavy infiltration in other organs. Radiation damage obviously reduced the capacity of the marrow not only to develop leukemia but also to support the growth of neoplastic cells.

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SUMMARY

Groups of chicks were inoculated with erythroleukemia virus 4, 8 and 20 days after exposure to 600 rad ^{60}Co radiation. Biopsy material from the femur marrow was obtained immediately prior to the virus inoculation. The incidence of leukemia and the latent periods to death of the chicks were recorded and a comparison is made between the groups of irradiated plus virus-inoculated chicks and, respectively, only virus-inoculated chicks. The results support the view that the pathogenic effect of the virus can be modified by varying the number of target cells and their differentiation.

ZUSAMMENFASSUNG

Gruppen von Küken wurden mit 600 rad ^{60}Co bestrahlt und 4, 8 und 20 Tage nach der Bestrahlung mit dem Erythroleukämievirus geimpft. Unmittelbar bevor der Einimpfung wurde Biopsiematerial aus dem Femurmark genommen. Die Untersuchungsergebnisse mit Hinsicht auf das Auftreten von Leukämie und die Latenzperioden bis zum Tode der Küken werden für die beiden Gruppen von bestrahlten und Virus-injizierten Küken bzw nur Virus-injizierten Küken in Relation gestellt. Die Ergebnisse deuten darauf hin dass der pathogene Effekt des Virus durch Veränderungen in der Anzahl von „Target-Zellen" und in ihrem Differenzierungsgrade modifiziert werden kann.

RÉSUMÉ

Des groupes de poussins ont été innoculés avec le virus de l'érythroleucémie quatre, huit et vingt jours après avoir été exposés à une dose de 600 rad de ^{60}Co; les poussins qui sont morts de leucémie ont été examinés histologiquement. Les auteurs ont comparé la fréquence de la leucémie et la période de latence jusqu'à la mort des poussins chez ceux qui avaient reçu l'irradiation et le virus et chez ceux qui n'avaient reçu que le virus. Les résultats de cette expérience viennent à l'appui de l'hypothèse que l'effet pathogène du virus peut être modifié au moyen de changements dans le nombre de cellules cibles et dans leur degré de différenciation.

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