Ca to such a cofactor. However, the strong, concentration-dependent inhibition by $La^{3+}$ of release reaction in washed cells makes it more likely that $La^{3+}$ does act on the cell, perhaps intracellularly. Quite recently O'BRIEN[19] also has shown $La^{3+}$ induced inhibition of platelet aggregation.

*Zusammenfassung.* $La^{3+}$-Ionen zeigten eine konzentrationsabhängige Hemmung der ADP-induzierten Aggregation menschlicher Blutplättchen. Diese Hemmung ist nicht von der Zahl der Zellen oder der Konzentration des ADP abhängig und lässt sich nicht durch Zusatz von $Ca^{2+}$-Ionen neutralisieren.

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## Effect of Normal and Irradiated Marrow Grafts on the Haemopoietic Recovery After a Sublethal Irradiation

A transitory accumulation of lymphoid-like cells in the bone-marrow after a sublethal irradiation has been reported by several authors[1–3]. These peculiar cells are called transitional cells[2,3] or X-cells[4].

In a previous report, we have shown that the lymphoid overshoot observed in mice after a 500 R irradiation[5] is not found in animals exposed to a lethal irradiation followed by a normal bone marrow graft[6]. This difference can be related either to the increase in the X-ray dose or to the presence of the injected marrow. In order to discriminate between these two possibilities, we decided to study the influence of a bone marrow graft in mice recovering from a 500 R irradiation.

*Methods.* Male RIII mice, 75 days old, were exposed to a 500 R X-irradiation under the following conditions: Siemens Stabilivolt apparatus, 190 KV, 18 mA, 0.5 mm Cu filter, 35 cm F.D., 210 R/min.

The mice were divided into 3 groups. 20 h after irradiation, the mice of the first group were injected i.v. with a suspension of normal isogeneic bone-marrow. The second group was treated as the first, except that the bone-marrow was harvested from donors irradiated at 500 R the day before. Each mice received $5 \times 10^6$ nucleated cells in 0.5 ml sterile Hanks solution. No additional treatment was given to the third group. In each experimental group, 4–6 animals were killed every other day from the 2nd to the 20th day. Additional mice were sacrificed at the 24th day.

The haematocrit and the leukocyte counts were measured. The total number of nucleated cells in 2 femurs was counted by means of an electronic counter. Marrow smears were prepared and stained by May-Grunwald-Giemsa method. The results of the myelograms were expressed in absolute number of cells for 2 femurs.

*Results.* The variations of the haematocrit, the leukocyte counts and the total number of marrow nucleated cells are closely similar in our 3 experimental models (Figure 1). The haematocrit is slightly decreased between the 4th and the 20th days. The leukocyte counts show a fast drop followed by a very slow recovery, the values found after 24 days being still below the control values. After a fall, during the first post-irradiation days, the total number of the marrow nucleated cells increases progressively; at the 20th day, normal values are reached again.

The results of the myelograms are plotted in Figure 2. The marrow injection does not influence the recovery of the myeloid and erythroid series. The differences concern the lymphoid-like cells. They rebound over the normal level from the 10th to the 14th day in the non-injected mice. This overshoot is also present in the animals grafted with irradiated marrow. No rebound can be detected after normal marrow injection.

*Discussion.* In 500 R irradiated animals, we reproduced the 'lymphoid' rebound already observed in previous experiments[15]. This overshoot was not modified by the administration of marrow irradiated 24 h prior to grafting. On the contrary, after a normal bone marrow graft, no lymphoid rebound could be produced.

Two mechanisms can be proposed to explain the absence of lymphoid overshoot. One could conceive that some cellular factor similar to the chalone exists in the normal

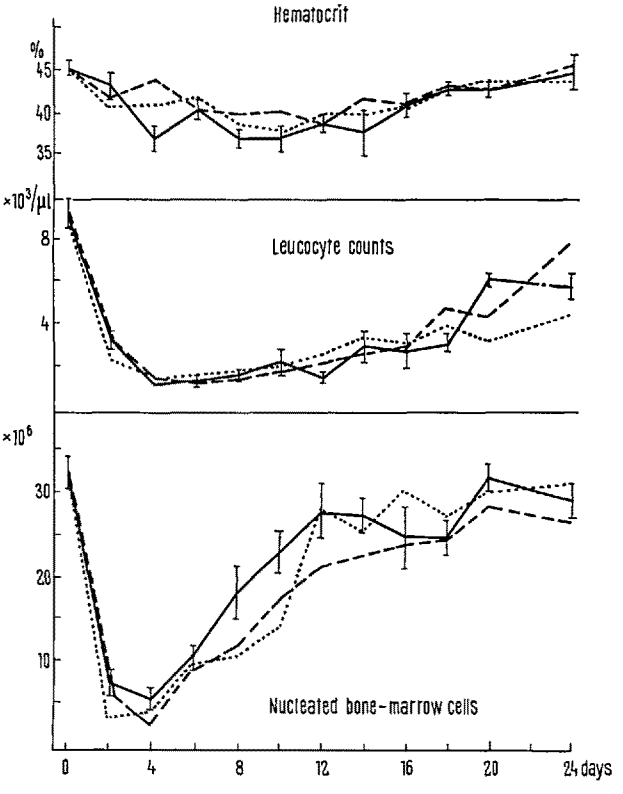


Fig. 1. Haematocrit values, leukocyte counts and bone marrow nucleated cell numbers from 2 femurs after a 500 R irradiation (——), a 500 R irradiation combined with a normal marrow graft (– – –) and a 500 R irradiation combined with an irradiated marrow graft (···).

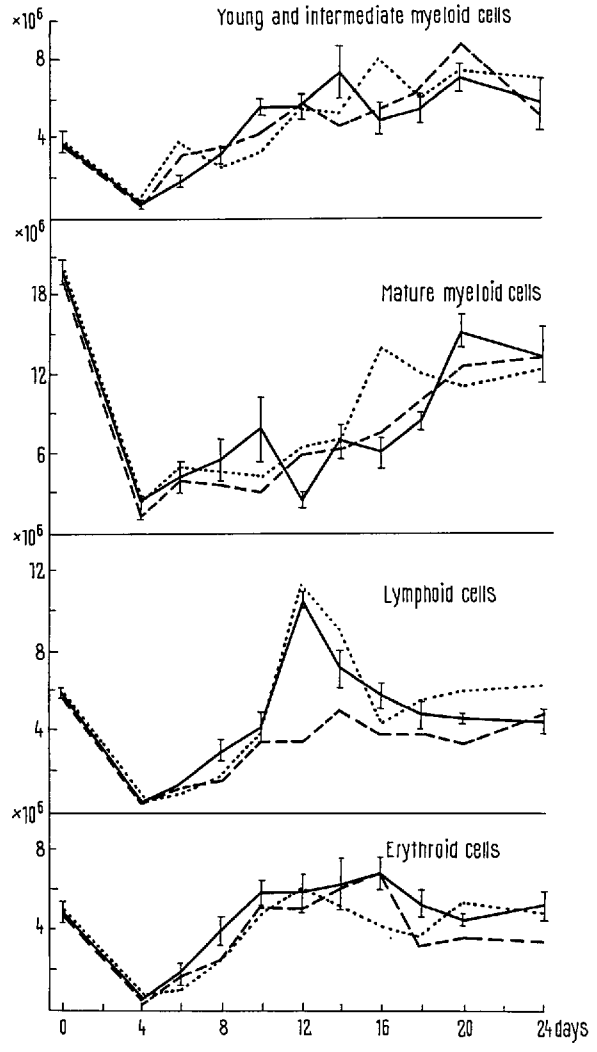


Fig. 2. Absolute numbers of various cell types after 500 R (——), 500 R with a normal marrow graft (– – –), and 500 R with an irradiated marrow graft (···).

marrow and is able to inhibit the development of the lymphoid population. This factor should be destroyed by the irradiation with a subsequent release of the lymphoid growth. A normal marrow graft would provide a sufficient amount of the inhibitory factor to restore its effect.

An alternative possibility is a competition between normal and irradiated marrows. The peculiar lymphoid (X cell) population, which constitutes an abnormal cell line in the adult marrow[4], should be overgrown by the normal corresponding cell line derived from the graft, i.e. from normal marrow which has been shown to recover without any lymphoid rebound[6].

However, the mechanisms regulating these cell populations are till now unknown. Thus other hypotheses can certainly not be discarded, even those involving extramedullary feed-back controls which could be perturbed by the irradiation and restored by a normal marrow graft.

*Résumé.* Après une irradiation sublétale, la mœlle présente une accumulation transitoire de cellules lymphoïdes particulières (cellules transitionnelles ou cellules X). Cette accumulation n'apparaît pas après une greffe de mœlle normale. Le comportement des cellules lymphoïdes n'est pas influencé par une greffe de mœlle irradiée.

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## 'High Dose' Immunologic Tolerance to *Escherichia coli* Lipopolysaccharide Assessed by Bacteriolytic and Hemolytic Plaque Assays

Immunologic tolerance or paralysis can be induced in experimental animals with highly immunogenic atigens derived from Gram-negative bacteria. Examples include the immunologic unresponsiveness induced in neonatal rodents to lipopolysaccharide (LPS) antigens of *Shigella paradysenteriae* and *Escherichia coli*, as well as to purified protein antigens derived from *Salmonella flagellin*[1–13]. Cellular aspects of tolerance to the LPS antigens have been investigated by means of a modified Jerne hemolytic plaque assay in agar gel, using target sheep erythrocytes coated with the appropriate bacterial extract[3,4,9–11]. Immobilization tests and bacterial adherence have been used to assess cellular events during tolerance to the flagellar antigens[6,12,13].

In studies with the *E. coli* LPS, tolerance has been achieved in both neonatal and adult mice[9–11,14–16]. Mice injected repeatedly as adults with the *E. coli* antigen showed a marked depression in their ability to respond to challenge immunization with this antigen, but not others[10,11]. However, experiments using both the passive hemolytic plaque assay, with antigen-sensitized sheep red blood cells, and a more direct bacteriolytic assay with viable bacteria as the indicator, resulted in contrasting results. A much greater suppression was evident when the passive hemolytic plaque assay was used to enumerate antibody forming cells[14–16]. In contrast, many more antibody-producing cells were found in the same spleen cell suspensions when the direct bacterial plaque procedure, with viable bacteria, was used as the indicator. Such findings were consistent with the concept that tolerance to antigens as complex as the somatic extracts of bacteria is most likely due to unresponsiveness to 'major' antigens present in the extract which may not sensitize the indicator erythrocytes[16].

In the earlier studies of BRITTON[10,11], tolerance was induced with relatively large concentrations of a detoxi-