Effects of the metals, Be, Fe, Cu and Al, on the mobility of immunoglobulin receptors

K. Morita, S. Inoue, Y. Murai, K. Watanabe and S. Shima

Department of Public Health, School of Medicine, Fujita-Gakuen University, Toyoake, Aichi 470-11 (Japan), and Department of Immunology, School of Hygiene, Fujita-Gakuen University, Toyoake, Aichi 470-11 (Japan), 15 July 1981

Summary. Cap formation in mouse spleen cells induced by antiimmunoglobulin was inhibited by the metals Be, Fe, Cu and Al. Be was especially strong as an inhibitor of cap formation. It is suggested that these metals might change the mobility of the membrane and have some biological effects on the cross association of antigen receptors when B lymphocytes are attached by them.

It is well known that B lymphocytes have immunoglobulin (Ig) receptors on their surfaces[1,2], and that the distribution of these membrane Igs may be visualized in a diffuse, patchy or capping pattern upon treatment with fluorescein-ated anti-Ig antibodies[3]. The influence of various lectins on cap formation has been studied[4–6], but reports on the effects of metals are very few. We have examined the effect of beryllium, iron, copper and aluminium on the mobility of lymphocyte Ig receptors.

Methods and results. Preparation of mouse spleen cells. Cells used for this experiments were spleen cells collected from ICR mice. Cells were preparated by the method of Yahara and Edelman[5] as follows. Spleens were teased in saline solution, pH 7.2 and filtered through a stainless-steel wire mesh. The filtrate was centrifuged at 800 rpm for 10 min. Pellets were washed twice with saline solution and exposed to distilled water for 30 sec to eliminate erythrocytes[7]. Cell suspensions were adjusted to a concentration of 2×10^7 cells/ml in saline solution.

Fluorescein-labeled anti-mouse Ig (FITC-anti-Ig). Antiserum was obtained by injecting rabbits i.m. with mouse IgG in complete Freund's adjuvant. The Ig fraction was isolated from the antiserum by precipitation with $(NH_4)_2SO_4$ at 38% saturation and by chromatography on DEAE-cellulose. Fractions eluted with the starting buffer (0.175 M sodium phosphate, pH 7.0) were dialyzed against water and lyophilized.

The resulting preparation was conjugated with fluorescein isothiocyanate (FITC) by the method of Cebra and Goldstein[8], and the ratio of fluorochrome-protein was 1.4.

Be, Fe, Cu and Al saline solution. $BeSO_4$, $FeSO_4$, $CuSO_4$, and $Al_2(SO_4)_3$ were dissolved in saline solution to give a concentration of 200 µM as metal and adjusted to pH 6.0–6.2.

Determination of percentage of capping cells with FITC-anti-Ig. FITC-anti-Ig was added to 2×10^7 spleen cells/ml in saline solution at a final protein concentration of 80 µg/ml, and then they were mixed. The mixture was incubated at 21 °C for 40 min and then washed 3 times with bovine serum albumin saline solution containing 10 mM NaN_3. The relative number of capping cells was observed by fluorescence microscopy and was usually of the order of 50%.

Effects of metals, Be, Fe, Cu and Al, on cap formation. After the cell suspensions had been treated with the various metal solutions at 21 °C for 10 min, FITC-anti-Ig was added and the mixture was further incubated at the same temperature for 4 min. The percentage of capping cells after treatment with each metal was determined by the following formula, and the corresponding values are shown in figure 1:

$$\frac{\text{capping cells after treatment with metal salts}}{\text{capping cells without metal salts}} \times 100$$

Tri-X film was used for photography, and exposure time was around 1 min. Several patterns of 'cap' and 'patch' are shown in figure 2.

The influence of the NaN_3 concentration and of the temperature on cap formation of Ig receptor on lymphocyte membrane were examined, and the results agreed well with those of other authors[4,5].

Discussion and conclusion. According to our present knowledge, membrane-associated Ig is thought to be floating between double membranes of lipid. Upon application of anti-Ig antibodies or antigen several Ig receptors apparently become associated with each other and form caps; cap formation proceeds through patching, and caps are eventu-

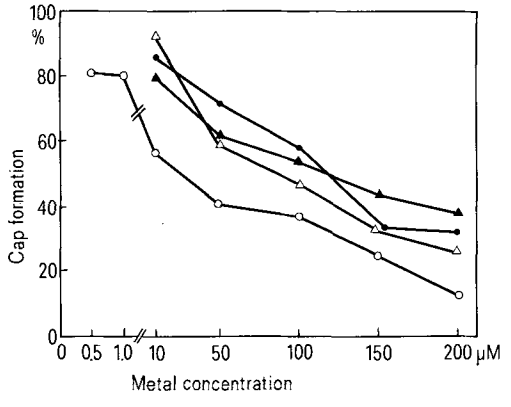

Figure 1. Percentage of capping cells after treatment of metals with FITC-anti-Ig. Be (○), Fe (●), Cu (▲) and Al (△).

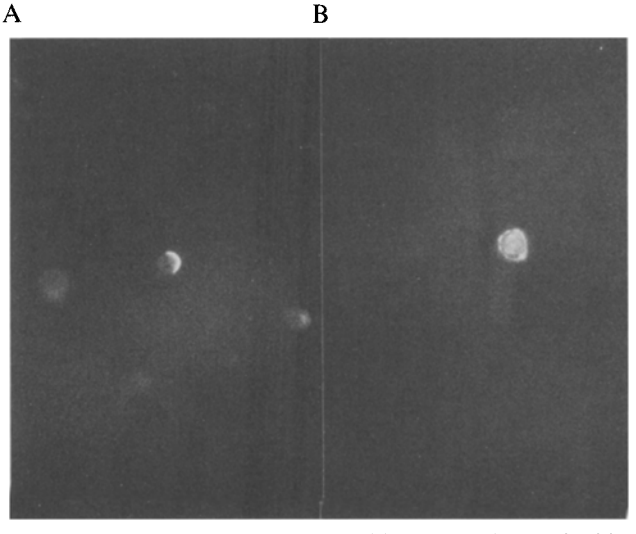

Figure 2. Labeling patterns of cells with FITC-anti-Ig and with beryllium. *A* Cap treated with FITC-anti-Ig (80 µg/ml) at 21 °C for 40 min. *B* Patch after beryllium was added.

ally pinocytosed. Patching occurs at low temperatures, but cap formation requires metabolic activity of the cells. It is not clear whether a metal such as Be, Fe, Cu and Al inhibits the metabolic activity of the cells or whether it prevents binding of anti-Ig to the membrane Igs. However, it is obvious from the present study that such metals inhibit the free diffusion of Ig receptors induced by anti-Ig. The inhibitory mechanism of metals on cap formation is now being studied. The following conclusions may be drawn from the study: 1. At 200 μM each metal tested inhibits cap formation to about 60%; and 2. beryllium, even at a concentration of only 0.5 μM, has a strong inhibitory effect on cap formation.

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Survival and endogenous spleen colonies of irradiated mice after skin wounding and hydroxyurea treatment[1,2]

G.D. Ledney, H.M. Gelston, Jr, S.R. Weinberg and E.D. Exum

Immunology Division, Experimental Hematology Department, Armed Forces Radiobiology Research Institute, Bethesda (Maryland 20814, USA), 17 February 1982

Summary. Wound trauma-induced survival from radiation may be related to increased mitosis in hematopoietic cells. This is supported by the cell cycle-dependent drug hydroxyurea, which 1. blocked survival of wounded mice injected 2 or 3 days after 900 rad and 2. reduced the number of endogenous CFU-s in wounded mice injected shortly before 700 rad.

Survival of mice after lethal irradiation is made possible by skin wounding at 24 h before lethal whole-body ^{60}Co irradiation[3,4]. Incidence of survival is reduced when the wound trauma follows irradiation[5,6]. Wounding before 700 rad seems to induce the appearance of colony-forming units-spleen (CFU-s) and granulocyte-macrophage colony-forming cells (GM-CFC) in the bone marrow and spleen before they appear in irradiated controls[3]. The percentage increases of exogenously derived CFU-s and GM-CFC were greater in the spleens of wounded animals than in their marrow compartments[3]. Even though the splenic values of the hematopoietic proliferative cells are greater than those of the marrow, the survival of skin-wounded, irradiated mice is independent of extramedullary splenic myelocytopoiesis[4]. While wound trauma may enhance the number of exogenous CFU-s in irradiated assay mice, such cells may not be responsible for the survival of individuals wounded before radiation[7]. This was suggested by exogenous and endogenous CFU-s studies using cell cycle-dependent drugs in irradiated mice[7]. This report, then, provides data from 2 experimental protocols using hydroxyurea, and those data support the idea that survival from lethal irradiation induced by prior skin wounding is related to the proliferation of endogenous (E) CFU-s.

Materials and methods. Female, 5-week-old (C57BL/6 X CBA) F1 Cum/BR mice from Cumberland View Farms, Clinton, TN, were quarantined for 2 weeks in groups of 15. The animals used were those from groups found to be free of *Pseudomonas* sp. and histologic lesions of common murine diseases. Experimental mice were housed 4 per sanitized cage, given Wayne Lab-Blox diet and chlorinated (10 ppm) water, and kept in controlled-environment rooms. Wounding and irradiation were performed on 14- to 16-week-old mice. Methyoxyflurane-anesthetized mice were wounded in the anterior dorsal skin fold and underlying panniculus carnosus muscle with a steel punch repeatedly cleaned by immersion in 70% ethanol. The wound was 2.0-2.5 cm^2, which was 4% of the total skin surface. Wounding was done 24 h before exposure to ^{60}Co, between 10.00 h and 14.00 h. The wounds were left untreated and open to the environment. Irradiated, non-wounded control mice were anesthetized before irradiation. All animals were exposed to whole-body radiation at a rate of 40 rad/min from bilateral ^{60}Co sources. In the 1st series of experiments, a total of 351 mice were used in survival studies (900 rad) and were observed for 30 days. The cell cycle-dependent drug hydroxyurea (HU) (Sigma Chemical, St. Louis, MO) was dissolved in sterile distilled water (50 mg/ml) and injected into mice i.p. (1 mg/g b.wt) at selected times before or after irradiation. 8 days after irradiation, mice were cervically dislocated and the number of splenic E-CFU-s counted. In preliminary studies we determined that 1. no 8-day E-CFU-s were detectable in mice given only 900 rad and 2. wounding before 900 rad resulted in only 1-2 E-CFU-s per spleen. While that number of E-CFU-s was sufficient to promote survival from 900 rad, we used 700 rad to better quantify E-CFU-s responses in combined injured animals treated with HU. Thus, in a 2nd series of experiments, E-CFU-s were determined in 400 HU-treated, skin-wounded mice 8 days after 700 rad. 3 replicate tests were done for each experimental protocol over a 6-month period. Since the responses in all replicates were similar, the data are combined in the figures.

Results. In the 1st series of experiments, 7 treatment groups were established. The 30-day survival fractions of 3 of these groups are shown in figure 1. HU injected into either nonwounded-irradiated or wounded-irradiated mice 16-h before irradiation resulted in 100% survival. However, injection of HU into nonwounded-irradiated mice shortly after exposure or at daily intervals for 5 days thereafter was associated with nearly 100% mortality. In mice wounded before irradiation, 40-95% of the animals died after injection with HU. These mortality rates are compared to the 10% mortality found in wounded-irradiated mice not given HU. Survival (after radiation) induced by skin-wound trauma was maximally blocked by treatment with HU on either day 2 or day 3 postirradiation. In the remaining four groups of control mice, all animals given 900 rad died, while mice either wounded only, injected with HU only, or injected with HU after wounding lived.