1st group are exposed to a single dose of 400 R whole body irradiation, those of the 2nd group to a 650 R dose. In each group, half the mice receive prior to irradiation a radioprotector treatment, while the other half is not protected. The radioprotection is performed as follows: 25 min before irradiation, the mice receive 15 mg glutathione orally; 10 min later, they receive i.p. 8.5 mg cysteine followed after 5 min by an i.p. injection of a mixture of 1.4 mg 2-β-aminoethylthiouroneum, 3 mg cysteamine and 0.65 mg 5-hydroxytryptamine[7]. All substances are dissolved in saline. Mice submitted to the mixture of chemical radioprotectors without any irradiation, and mice free of any treatment, represent the control groups. 6 animals of the different experimental modalities are sacrificed every other day from the 2nd to the 16th day and the 20th and 24th days. The variations of the total number of nucleated bone marrow cells and of the lymphoid marrow cells in 2 femurs are scored. The changes in the number of thymic cells are also determined. Our counting techniques have been previously described[8].

Results. After an irradiation of 400 R (figure 1), the marrow nucleated cells are strongly reduced in number between the 2nd and the 4th day; the recovery is fast, with an overshoot at the 14th day; the number of lymphoid marrow cells varies in the same way, but the rebound over the normal level is observed at the 12th day. The administration of radioprotectors prior to the irradiation prevents partially the decrease of the marrow nucleated cells and has little influence on the marrow repopulation. On the contrary, the chemical protectors have only a slight effect on the destruction of the lymphoid marrow cells, but they increase the regeneration with the development of an especially important lymphoid rebound; the mixture of chemical protectors without irradiation does not significantly modify the bone marrow populations.

The modifications of the thymic cell number after a 400 R irradiation, with or without protection and after the administration of chemical protectors only, are also plotted in figure 1. The irradiation induces an important reduction of the thymic cell population; the recovery begins at the 4th day and reaches, on the 12th day, a level clearly lower than the normal values. A secondary atrophy is observed from the 14th day to the end of the observation period. The protectors do not modify the thymic regeneration; they have only a little effect on the secondary atrophy. Indeed, on day 24, the number of thymocytes is significantly higher in protected animals. The administration of the protectors mixture without any irradiation induces a fall of the thymic cell number lasting 6 days (50% of the normal value); the thymic regeneration presents a transient phase of proliferation with a return to the normal level at the 14th day; the regeneration is also followed by a secondary atrophy which is still present at the end of the observation period.

Figure 2 illustrates the modifications of the nucleated bone marrow cells, lymphoid marrow cells and thymic cells after an irradiation of 650 R with or without chemical protection. The evolution of these different populations is rather similar to what is observed after a 400 R dose, but the marrow is not wholly repopulated by the 24th day, the lymphoid peak is less important and the thymic secondary atrophy more pronounced. The protection allows a fast and complete restoration of the nucleated marrow cells and greatly increases the lymphoid rebound. It has no effect on the primary thymic recovery but it reduces the secondary atrophy as early as the 14th day. All the modifications shown here are statistically significant.

Discussion. The sole administration of the mixture of chemical protectors has very little effect on the bone marrow but it induces an important decrease of the thymocytes number. The administration of the same mixture of chemical protectors before the irradiation permits a better bone marrow repopulation and reduces the late thymic atrophy (condition which precedes the apparition of thymic lymphomas). Contrarily to our expectations, the lymphoid peak is considerably increased in radioprotected animals. We are thus confronted with a situation where a lymphoid rebound appears in the bone marrow, whereas, as demonstrated by others[5,6], the development of lymphomas is reduced. However, the composition of this rebound may be quite different from that observed after an irradiation without any chemical protection. Indeed, after irradiation, the bone marrow contains peculiar lymphoid blast cells, called X cells, which have been also observed in the thymus and have been suspected of playing an important role in leukemogenesis. Further ultrastructural investigations are necessary to verify whether X cells are also present in the animals chemically protected.

1 H.S. Kaplan and M.B. Brown, J. nat. Cancer Inst. *13*, 185 (1952).
2 L.J. Simar, J. Haot and E.H. Betz, Eur. J. Cancer *4*, 529 (1968).
3 J. Boniver, M. Delrez, L.J. Simar and J. Haot, Beitr. Path. *150*, 229 (1973).
4 M. Delrez, J. Haot and E.H. Betz, Experientia *27*, 453 (1971).
5 J.R. Maisin, Proc. Symp. Radiat. Cancer.
6 J.R. Maisin, A. Decleve, G.B. Gerber, G. Mattelin and M. Lambiet-Collier, in press.
7 J.R. Maisin, G. Mattelin, A. Fridman-Manduzio and J. van Paren, Radiat. Res. *35*, 26 (1968).
8 J. Haot and N.F. Barakina, Acta haemat. *42*, 347 (1969).

Relationship between lymphoid cell population and levels of cholesterol or phospholipids

S. Kigoshi

Department of Pharmacology, School of Medicine, Kanazawa University, Kanazawa 920 (Japan), 17 October 1977

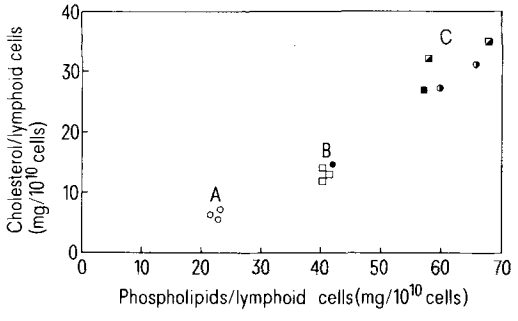
Summary. Lymphoid cells obtained from mouse thymus were divided into 3 groups according to the levels of free cholesterol and phospholipids.

The structural lipids of mammalian cell membrane are known to consist primarily of cholesterol and phospholipids, of which the proportions and structure are very important for the properties and functions of the cell membrane[1-4]. Studies on the lymphocyte lipids indicated differences in the cholesterol levels between normal and leukemic cells from man or animals[5-7]. However, relatively little is known about the relation of the lipid levels and various lymphocyte populations. In man and animals, there are 2 major lymphocyte populations (thymus-derived and bone marrow-derived lymphocytes), which are further divided into subpopulations[8]. In this study we have ex-

Contents of free cholesterol and phospholipids in total and cortisone-resistant lymphoid cells from thymus or mesenteric lymph node of control mice and animals treated with lipopolysaccharide from *E. coli* (LPS) or a hemolytic streptococcus (cocci)[a]

Tissues	Lymphoid cells[b]	Adjuvants	Lipid contents in lymphoid cells ($mg/10^{10}$ cells)[c] Cholesterol	Phospholipids	Cholesterol Phospholipids (molar ratio)[d]
Thymus	Total cells	(control)	6.7 ± 0.2	23.1 ± 0.7	0.58
		LPS	6.1 ± 0.3	22.4 ± 0.6	0.55
		Cocci	5.9 ± 0.3	22.1 ± 0.4	0.54
	Cortisone-resistant cells	(control)	14.6 ± 0.3	41.4 ± 1.0	0.71
		LPS	27.2 ± 1.3	59.5 ± 1.2	0.92
		Cocci	31.0 ± 1.0	65.7 ± 1.8	0.94
Mesenteric lymph node	Total cells	(control)	12.5 ± 0.3	40.4 ± 0.7	0.62
		LPS	13.4 ± 0.3	40.7 ± 1.1	0.66
		Cocci	14.1 ± 0.6	40.7 ± 1.1	0.69
	Cortisone-resistant cells	(control)	26.8 ± 0.7	56.7 ± 1.0	0.95
		LPS	35.0 ± 1.0	67.8 ± 1.6	1.03
		Cocci	32.5 ± 2.7	57.6 ± 2.5	1.13

[a] Mice were injected i.p. with 50 µg of LPS or 5 mg of dried streptococcal cells per mouse and killed 14 days later. Control animals were given saline alone. [b] Cortisone-resistant cells and total cells were prepared from mice treated with or without hydrocortisone acetate (12.5 mg/100 g of b.wt) 2 days before sacrifice. [c] Each value represents mean ± SE of 10 experiments, except the cortisone-resistant cells from control mice (n = 15). [d] The phospholipid mol.wt was assumed to be 775.



Relationship between the levels of free cholesterol and phospholipids in lymphoid cells from thymus (○) or mesenteric lymph node (□) of mice. Each of the cholesterol value in lymphoid cells (table) was plotted against the corresponding value of phospholipids. Lymphoid cells examined were separated into 3 groups (group A, B and C) according to the lipid levels. Contents of cholesterol and phospholipids (mg of lipids/10^{10} cells) in each group of lymphoid cells were as follows: 6.2±0.2 and 22.5±0.3 for group A (n = 30), 13.7±0.2 and 40.9±0.4 for group B (n = 45), 30.1±0.7 and 61.0±0.9 for group C (n = 55). Symbols: ○ and □, total cells; ● and ■, cortisone-resistant cells from control mice; ◑ and ◪, cortisone-resistant cells from adjuvant-treated animals.

amined the amounts of free cholesterol and phospholipids in the total and cortisone-resistant lymphoid cells from thymus and mesenteric lymph node of mice treated with or without adjuvants. It was found that the lymphoid cells examined may be divided into 3 groups according to the lipid levels.

A total of 50 female mice (ddN strain, 8–9 weeks old) in each group were pretreated with the following adjuvants: lipopolysaccharide from *E. coli*[9] 0111: B4, Westphal method (Difco Laboratories) and a hemolytic streptococcus (group A, Su strain)[10,11]. Streptococcal cells[12] were treated with 30 volumes of acetone at room temperature for 24 h[13], dried in vacuo and suspended in physiological saline. Groups of mice were injected i.p. with 0.5 ml of saline containing 50 µg of lipopolysaccharide or 0.5 ml of streptococcal suspension (10 mg/ml) per mouse, and killed by cervical dislocation 14 days later. Animals given saline alone were also used in this study (control mice); 2 days before sacrifice, some groups of adjuvant-treated mice and control animals were injected i.p. with 12.5 mg of hydrocortisone acetate (Shering AG) per 100 g of b.wt[14]. Suspensions of lymphoid cells were prepared from thymus and mesenteric lymph node of mice treated with hydrocortisone or of controls, following the procedure described previously[15]. The cell suspensions contained 92–95% of lymphocytes and 3–6% of macrophages. Extraction and quantitation of the lymphoid cell lipids were performed by the method reported elsewhere[16]. In this paper, lymphoid cells prepared from mice with and without treatment by hydrocortisone are referred to as cortisone-resistant cells and total cells, respectively.

In the thymic lymphoid cells from mice, a marked difference was found in the lipid contents between total and cortisone-resistant cells or between cortisone-resistant cells from control and adjuvant-treated animals (table). The amounts of cholesterol and phospholipids in the total thymic lymphoid cells were 6–7 mg and 22–23 mg, whereas the lipid contents in the cortisone-resistant thymic lymphoid cells were 15 mg and 41 mg for control mice or 27–31 mg and 60–66 mg for adjuvant-treated animals ($p < 0.01$). As compared with the total lymphoid cells from thymus, the corresponding cells from mesenteric lymph node of mice contained high levels of cholesterol and phospholipids (13–14 mg and 40–41 mg). However, with regard to lipid levels total mesenteric lymphoid cells were similar to the cortisone-resistant thymic lymphoid cells from control mice. Furthermore, the difference between the lipid contents of cortisone-resistant thymic lymphoid cells of adjuvant-treated mice and of lymph node cells was small (27–31 mg and 60–66 mg in thymic cells, 27–35 mg and 57–68 mg in mesenteric cells). Therefore, lymphoid cells from thymus and mesenteric lymph nodes of mice may be grouped according to the levels of cholesterol or phospholipids. When plotting cholesterol values of lymphoid cells against corresponding values of phospholipids (figure), the cells examined are separated into 3 groups (group A, B and C) according to their lipid levels. Cortisone-resistant thymic lymphoid cells from control mice and the total mesenteric lymphoid cells belonged to one group (group B), and cortisone-resistant lymphoid cells from thymus and lymph nodes of adjuvant-treated animals into another group (group C). In contrast, group A included the total thymic lymphoid cells alone. The contents of cholesterol and phospholipids in each group of lymphoid cells were as follows: 6.2 mg and 22.5 mg for group A, 13.7 mg and 40.9 mg for group B, 30.1 mg and 61.0 mg for group C ($p < 0.01$). This separation of lymphoid cells into groups shows the presence of 2 distinct populations of cortisone-resistant thymic lymphoid cells.

The results described above indicate that the lymphoid cells obtained from mouse thymus may be divided into 3 groups according to the levels of free cholesterol and phospholipids: 1 group of total cells and 2 groups of cortisone-resistant cells, one group of which was obtained from control mice and another group of the cells from adjuvant-treated animals. It is widely accepted that within mouse thymus the cortisone-resistant lymphocytes (small population, about 5%) are mature cells and the cortisone-sensitive lymphocytes (large population, about 95%) are immature cells[17,18]. In this study, the group containing cortisone-resistant lymphoid cells from mouse thymus amounted to about 6% of total lymphoid cells. This proportionality suggests a similarity in the lipid composition between total and cortisone-sensitive thymic lymphoid cells. Cortisone-resistant lymphoid cells from the thymus of adjuvant-treated mice and from the lymph node of control animals belonged to the same group. The cortisone-resistant lymphocytes from mouse lymph node were reported to be almost exclusively thymus-derived cells[8,18]. In normal mice, therefore, there may be 3 groups of the lymphocytes derived from thymus: 1 group of immature cells and 2 groups of mature cells, such as the cortisone-resistant cells from thymus or mesenteric lymph node. The cortisone-resistant mesenteric lymphoid cells from control mice appear to be more mature than the corresponding thymic cells, since the cholesterol to phospholipid molar ratio was 0.95 for mesenteric cells and 0.71 for thymic cells, respectively[5-7]. Interestingly, the thymus-derived lymphocytes from mouse lymph node were shown to be separable into 2 subpopulations by cell electrophoresis, which indicated the presence of 3 subgroups of lymphocytes (1 group of immature cells and 2 groups of mature cells) in mouse thymus[19,20].

1 S.J. Singer and G.L. Nicolson, Science *175*, 720 (1972).
2 A.D. Bangham, A. Rev. Biochem. *41*, 753 (1972).
3 M.S. Bretscher, Science *181*, 622 (1973).
4 S.J. Singer, Adv. Immun. *19*, 1 (1974).
5 E.L. Gottfried, J. Lipid Res. *8*, 321 (1967).
6 I. Vlodavsky and L. Sachs, Nature, Lond. *250*, 67 (1974).
7 M. Inbar and M. Shinitzky, Proc. nat. Acad. Sci. USA *71*, 4229 (1974).
8 M.F. Greaves, J.J.T. Owen and M.C. Raff, T and B Lymphocytes: Origin, Properties and Roles in Immune Responses. Excerpta Medica, Amsterdam 1973.
9 O. Westphal, Int. Archs Allergy appl. Immun. *49*, 1 (1975).
10 N. Kaibara, Jap. J. exp. Med. *44*, 399 (1974).
11 I. Azuma, K. Sugimura, T. Taniyama, M. Yamawaki, Y. Yamamura, S. Kusumoto and T. Shiba, Infect. Immunity *14*, 18 (1976).
12 S. Kigoshi, Experientia *17*, 976 (1971).
13 L. Chadid, A. Lamensans, F. Parant, M. Parant, A. Adam, J.F. Petit and S. Leder, Cancer Res. *33*, 2167 (1973).
14 J.J. Cohen, M. Fischbach and H.N. Claman, J. Immun. *105*, 1146 (1970).
15 S. Kigoshi and M. Akiyama, Experientia *31*, 1225 (1975).
16 S. Kigoshi and R. Ito, Experientia *29*, 1408 (1973).
17 M.C. Raff and H. Cantor, Progress in Immunology, p. 83. Ed. B. Amos. Academic Press, New York 1971.
18 H.N. Claman, New Engl. J. Med. *287*, 388 (1972).
19 K. Zeiller, G. Pascher, G. Wagner, H.G. Liebich, E. Holzberg and K. Hanning, Immunology *26*, 995 (1974).
20 F. Dumont, Immunology *26*, 1051 (1974).

Modification of lymphocyte response to phytomitogens by polycations and polyanions

B. Larsen and I. Heron

The Institute of Cancer Research, Radiumstationen, DK-8000 Aarhus C (Denmark), and The Institute of Medical Microbiology, University of Aarhus, DK-8000 Aarhus C (Denmark), 13 February 1978

Summary. The stimulating effect on mitogen-induced lymphocyte response by polycationic compounds such as polylysine, DEAE dextran protamine and methylated albumin, is studied at different serum-protein concentrations and mitogen concentrations. It is suggested that the polar interaction between polycationic compounds and glycoproteins are of major importance for reactivity of lymphocytes to mitogens.

Normal serum is known to contain glycoproteins that interfere with the in vitro response of lymphocytes to phytohaemagglutinin (PHA) and concanavalin A (Con A)[1]. The α_2-glycoprotein fraction[2,3] prepared from normal human serum exhibits immunosuppressive effects, and likewise transcortin from normal serum inhibits the lymphocyte response to PHA[4]. From foetal serum, α-foetoprotein can be prepared in large amounts. This glycoprotein, which is also present in sera from patients with primary liver cancer[5], likewise has an immunosuppressive effect.

Serum-protein components with immunosuppressive activity are found in cancer patients and experimental animals bearing transplantable tumours. Transcortin is greatly increased in patients with breast cancer[4], and several inhibitory protein fractions and blocking factors are demonstrable in the serum from patients with various malignant diseases[6].

Characteristic of this group of α-glycoproteins is their relatively high sialic acid content which supplies the proteins with negatively charged groups. Focusing on the anionic nature of the immunosuppressive glycoproteins leads to the assumption that reduction of the negative charge by treatment with neuraminidase or polar interactions with polycations interferes with their effects. Treatment of rat lymph-node lymphocytes with neuraminidase, or addition of the polycations poly-L-ornithine or poly-D-lysine, enhances their response to PHA or Con A. This indicates that surface charge of lymphocytes is of importance for their mitogen-induced transformation[7]. Similarly, the consequence of polar interactions between anionic and cationic components in the cellular micro-environment needs consideration in order to try to estimate the extent of immunosuppressive activity in serum.

Materials and methods. Peripheral blood lymphocytes from normal volunteers were isolated by Ficoll-Isopaque flotation. The cells were cultured in medium Tc 199 supplemented with 2-18% heat-inactivated pooled normal human serum, 100 units ml^{-1} penicillin, 100 $\mu g\ ml^{-1}$ streptomycin, and 20 μmoles ml^{-1} HEPES buffer; 2×10^5 cells in 0.2 ml of the medium were added per well in Nunc microtitre