Table I

Experiment	Influence of temperature on TCT-binding by plasma-protein (%) 37 °C	25 °C
1	79	98
2	83	100
3	82	97
4	85	100

Table II

Amount of cold TCT added (μg)	TCT-I^{125} binding to plasma protein at 25 °C (%)
25	78
50	35
75	10
100	0

amidoblack to detect the binding proteins (Figures 1 and 2), with the fingerprint technique. Their identification was performed using monospecific immunosera, anti-α_1-antitrypsine, antitransferine, anti-α_1-lipoproteine, anti-α_2-macroglobuline, anti-IgA, anti-IgG anti-IgM. Furthermore plaques were stained after exposure to polyspecific immunosera anti-lipoproteins, -glycoproteins, -haptoglobuline and -hemopexine. Human γ-globuline, precipitated by addition of sodium sulfate, was used for two-dimensional electrophoretic separation.

Results. 1. *Equilibrium dialysis:* TCA precipitated initially 80% of labelled H-CT and 25% of total radioactivity after dialysis (72 h). At 37 °C, 80 to 85% of H-CT were bound to plasma proteins; at 25 °C, the binding was nearly complete (Table I). The results were the same when amount of added H-CT changed from 300 to 3 ng. Addition of increasing amounts of cold H-CT to the plasma decreased the binding of labelled H-CT (Table II) which became undetectable in presence of 100 μg cold H-CT.

2. *Two-dimensional radioimmunoelectrophoresis:* At the dose level of 250 ng/ml, H-CT radioactivity was bound to a single protein peak, which was later identified as IgM-globuline (β_2-macroglobuline) with specific anti IgM immunoserum. At the higher dose level (1 μg/ml), several other proteins bound radioactivity: albumine, α_2-macroglobuline and α_2-lipoproteine (Figures 1 and 2).

Discussion. 1. The results presented demonstrate an increase of H-CT binding to plasma proteins with decreasing temperature. At 37 °C, 80% of H-CT is bound as assessed by equilibrium dialysis.

2. Addition of cold H-CT decreases the amount of bound H-CT: the displacement is in favour of a similar binding of labelled and cold H-CT and makes unlikely alternation of H-CT during the labelling procedure, at least at the binding site. Gel filtration and preparative ultracentrifugation were used by LEGGATE et al.[6] to study interactions of H-CT with plasma proteins; they found evidence for an interaction but did not identify specific binding proteins.

3. The amount of circulating CT in man is controversial. We have matched the concentrations of H-CT with the one detected in the hog. In most equilibrium dialysis experiments, H-CT was added in amounts equivalent to concentrations prevailing in thyroid venous plasma (150 ng/ml). In a few instances, 1.5 ng/ml were used in order to come closer to systemic circulating hormone levels. The ratio bound/free H-CT is identical in both cases, indicating that plasma proteins can display a binding capacity able to match the high hormone concentration of effluent thyroid blood[7].

Résumé. La protéine porteuse spécifique de la CT-H est l'IgM lorsque la teneur en hormone est relativement faible (jusqu'à 250 ng/ml de plasma). Lorsque la concentration augmente, la CT-H se lie aux α_2-macroglobulines, α_2-lipoprotéines et à l'albumine, dont l'affinité pour l'hormone est moindre mais la capacité de liaison importante. Il est logique d'admettre que des anomalies portant sur les protéines porteuses spécifiques modifient la distribution et le métabolisme de la CT, ce qui représenterait un mécanisme pathophysiologique nouveau de dyscalcitonie humaine.

G. MILHAUD and I. SZLAMKA

Laboratoire des Isotopes, Laboratoire associé au C.N.R.S., Faculté de Médecine Saint-Antoine, 27, rue Chaligny, F-75 Paris 12e (France), 1 June 1971.

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Cytotoxic Potential of Lymphocytes Stimulated with Autochthonous Lymphoid Cell Line Cells

Human lymphocytes are activated by contact with histocompatibility antigens on the lymphocytes of another donor. The reaction, which occurs with lymphocytes of all normal individuals and with lymphocytes from cord bloods, requires no prior immunisation of the cell donors with the antigens concerned (for refs. see[1]). An immunological basis for the reaction is indicated, however, by the demonstration, in rats, that lymphocytes from tolerant animals are specifically unresponsive to cells of the tolerance inducing strain[2,3]. Human lymphocytes are also activated by cells from human lymphoblastoid cell lines (LCLs) established from the blood cells of normal individuals and patients with various disorders. The reaction is essentially similar to a 'one-way' mixed lymphocyte reaction but of greater intensity[4]. Lymphocytes activated by culture with X-irradiated cells from a particular cell line are cytotoxic to these cells and the cells of other lines[5].

New surface antigens on the LCL cells as well as histocompatibility antigens must be capable of activating lymphocytes since cells from autochthonous LCLs are stimulatory[6-9], although if the conditions are carefully controlled, to a lesser degree than cells from a histoin-

Table I. Stimulation of lymphocytes by cells from an autochthonous LCL and their subsequent cytotoxic action

Composition of culture	Stimulation (^{3}H-thymidine incorporated dpm)	Cytotoxicity versus: ^{51}Cr-DEW-1 (% released)
ED	6,134	18.8
ED + X ED	3,243	19.4
ED + X DEW-1	23,526	31.5
ED + X FLE-1	42,887	26.7
ED + X SAD-1	41,504	21.9
DH	1,522	20.7
DH + X ED	3,506	25.6
DH + X DEW-1	32,145	40.6
DH + X FLE-1	32,934	35.2
DH + X SAD-1	34,096	37.5
Controls		
ED rbc + X ED	173	19.2
ED rbc + X DEW-1	1,767	19.3
ED rbc + X FLE-1	178	19.2
ED rbc + X SAD-1	208	19.0

ED, blood lymphocytes from patients recovered from infectious mononucleosis 6 months before the experiment. DH, blood lymphocytes from normal individual. ED rbc, red blood cells. DEW-1, LCL established from a blood sample from patient ED taken during the illness. FLE-1 and SAD-1, two LCL from unrelated individuals. X, x-irradiated. dpm, disintegration per minute. Autochthonous mixture underlined. Lymphocytes were cultured for 6 days at 1×10^6/ml in $3 \times 1/2$ inch capped tubes. 10^5X-irradiated LCL cells were added where indicated. To study the cytotoxicity 0.5 ml fluid was removed on day 6 and 10^6 ^{51}Cr labelled DEW-1 cells added in 0.5 ml fresh medium. After 6 h the tubes were centrifuged (500 *g*, 5 min) and the supernatants carefully transferred to counting vials. The deposits were dissolved in 0.1 ml N NaOH and transferred to vials. Results are expressed as the percentage of the total activity added of ^{51}Cr released into the supernate. To assess the stimulation 0.5 μCi ^{3}H-thymidine was added on day 6 and the cells harvested on day 7 (ref. [12]). All values are means of triplicate cultures.

compatible line (ref.[6] and unpublished observations). The neo-antigens may arise coincidentally with the change of the lymphoid cell to a continuously growing state resulting in the establishment of the LCL. This could be the result of a 'spontaneous' mutation or of a mutation induced by an EB virus which is known to be present in most, if not all, of the LCLs[10,11]. If the activation of lymphocytes in this system is part of an immunological reaction in which the cells bearing neo-antigens are recognised as 'non-self', it should result in the generation of effector cells cytotoxic to the autochthonous LCL cells. This paper described such a reaction.

Lymphoid cell lines established at various times during the period 1968–1970 from the blood cells of patients in the acute phase of infectious mononucleosis[11] were maintained routinely in F10 medium supplemented with 20% foetal bovine serum, 10% tryptose phosphate broth, penicillin and streptomycin. Tests for the presence of mycoplasma infection were regularly performed and found to be negative. For use as stimulants the LCL cells were exposed to 3000 rads of X-irradiation at a cell concentration of 2×10^6/ml[6]. For use as target cells they were labelled

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Table II. Stimulation of lymphocytes by autochthonous LCL cells and their subsequent cytotoxicity against autochthonous and allogeneic LCL cells

Composition of culture	Stimulation (^{3}H-thymidine incorporated, dpm)	Cytotoxicity versus: ^{51}Cr-YUD-1A (% released)	^{51}Cr-GOL-1P (% released)
KY	1,511	8.7	16.4
KY + X KY	817	7.7	14.3
KY + X YUD-1A	47,727	17.5	16.0
KY + X YUD-1E	49,272	16.8	16.5
KY + X GOL-1P	38,281	14.5	23.0
DH	1,785	8.9	15.0
DH + X KY	9,500	8.3	16.1
DH + X YUD-1A	45,854	16.6	19.1
DH + X YUD-1E	34,450	14.6	19.1
DH + X GOL-1P	37,227	11.3	20.4
Controls			
KY rbc + X KY	185	9.2	14.7
KY rbc + X YUD-1A	1,396	8.4	14.9
KY rbc + Y XUD-1E	8,262	9.3	14.2
KY rbc + X GOL-1P	300	8.7	15.2

KY, blood lymphocytes from patient recovered from infectious mononucleosis 7 months before the experiment. DH, blood lymphocytes from normal individual. KY rbc, red blood cells. YUD-1A and YUD-1E, 2 sub-lines established independently from the same blood sample taken from KY during the illness. GOL-1P, LCL from unrelated individual. Autochthonous mixtures underlined. Other details as for Table I.

with ^{51}Cr by incubation for 24 h with $Na_2{}^{51}CrO_4$ (1 μCi/10^6 cells/ml).

Mitotic activation of fresh blood lymphocytes (10^6/ml in 20% pooled human serum gelatin/Eagles MEM) incubated with X-irradiated LCL cells (added to give a final concentration of 10^5 cells/ml soon after X-irradiation) was assessed by the incorporation of thymidine-[methyl-^{3}H] 0.5 μCi; 150 mCi/mmole) into the trichloroacetic acid-insoluble fraction over the interval day 6–7. Details of the methods are to be found in refs. 4 and 12. Suitable controls lacking X-irradiated LCL cells or fresh lymphocytes were treated identically.

The cytotoxic capacity of the activated blood lymphocytes was assessed by the chromium release technique. Control cultures contained donor red cells and either blood lymphocytes of the donor or the X-irradiated LCL cells used as stimulant but not both[5]. In each experiment the response of blood lymphocytes from an unrelated donor as well as from the original LCL donor were compared. As a further control (to cover any possible change induced by X-irradiation) X-irradiated blood lymphocytes were cultured with autochthonous or allogeneic blood lymphocytes.

Stimulation (enhanced ^{3}H-thymidine incorporation) induced by autochthonous LCL cells occurred with the lymphocytes from all six patients studied. The activated lymphocytes were cytotoxic to ^{51}Cr-labelled autochthonous and allogeneic cells. The results of two typical experiments are shown in Tables I and II. The stimulation and cytotoxicity of the lymphocytes in the autochthonous situation was not a result of changes induced by the X-irradiation since X-irradiated blood lymphocytes did not stimulate autochthonous lymphocytes nor was it due to foetal calf serum antigens in the culture fluid since human serum was used as the serum supplement in all experiments. Moreover, GREEN and SELL[9] have reported that foetal calf serum antigens were not important in their autochthonous stimulations.

It may be concluded that neo-antigens on the LCL cells initiate a lymphocyte stimulation, in vitro, resulting in the death of the LCL cells. The fact that allogenic as well as autochthonous LCL cells can be killed by lymphocytes stimulated with autochthonous LCL cells suggests that either the killing phase is of limited specificity or that there are cross reacting antigens shared by the various LCL cells. These points are currently under investigation.

If it is accepted that 'deviant' lymphocytes, similar to LCL cells, may arise in vivo as a result of viral transformation or other cause, the in vitro phenomena described must closely mimic an immunological surveillance mechanism operative in the host.

Résumé. Les petits lymphocytes du sang, après avoir été mis en contact pendant plusieurs jours avec les cellules lymphoïdes d'une lignée autochthone, peuvent tuer les cellules de cette lignée et d'autres lignées. Ces investigations peuvent apporter de nouvelles données sur l'opération de contrôle immunologique.

D. A. HARDY and C. M. STEEL[13]

Department of Experimental Pathology, University of Birmingham, Birmingham B15 2TJ (England); and University Department of Medicine, and M.R.C. Clinical and Cytogenetics Unit, and Western General Hospital, Edinburgh (Scotland),
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Immunization against Cobra Venom

Active immunization of experimental animals against snake venoms is hampered by two features: first, the toxicity precludes application of high initial doses; second, the main toxic principles, against which it is imperative to obtain high antibody titres, are poor antigens because of their low molecular weight[1].

By cross-linking cobra venom with glutardialdehyde we have been able to circumvent both these difficulties. The toxicity was profoundly reduced, whereas good antibody formation against the toxins was observed. It seems possible to use this procedure for the routine immunization of antiserum-yielding animals, and it may even be adaptable to the active immunization of humans.

Naja naja venom was treated in the following manner. A 2% toxin solution in 0.1 *M* phosphate buffer, pH 6.8, was prepared and 1% glutardialdehyde solution (0.01 ml/mg venom) was added under constant stirring. The mixture was allowed to react for 2 h at room temperature. After emulsifying with an equal volume of complete Freund's adjuvant, the preparations were immediately injected i.m. or s.c. into rabbits.

By the treatment with glutardialdehyde, the toxins were largely inactivated. Whereas all of 3 injected rabbits (3 kg) died after 2 mg genuine venom (in Freund's adjuvant), in the course of a few hours, all of 10 animals survived 20 mg cross-linked venom, without symptoms of adverse effects. Two rabbits died after 40 mg.

Two rabbits (3 kg) received 10 mg cross-linked venom. They were boosted 3 times with the same and twice with the double amount. After $3^1/_2$ months venous blood was drawn from the ear vessels and the serum checked by agar-immune electrophoresis. Both antisera developed several precipitation lines against the components of whole cobra venom, among them the basic toxins (Figure, upper trough), as well as against purified preparations of direct lytic factor (DLF[2]) and phospholipase A.

The toxin-neutralizing potency of 1 of the 2 antisera was investigated in mice. The LD_{50} of the genuine venom

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