Proc. Estonian Acad. Sci. Biol., 1993, 42, 3, 165—169
https://doi.org/10.3176/biol.1993.3.01

GENERATION OF OXYGEN RADICALS BY INTACT AND IRRADIATED THYMOCYTES

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Presented by U. Margna

Received November 16, 1992; revised February 11, 1993; accepted February 26, 1993

Abstract. The generation of oxygen radicals in thymocytes induced by their adhesion to glass or nylon was studied by the method of chemiluminescence. Gamma-irradiation of thymocytes stimulated their system of generation of oxygen radicals; the maximum effect was established at the doses of 2...5 Gy. In the cells of the bursa of Fabricius similar response to adhesion or irradiation was not observed.

Key words: thymocytes, irradiation, chemiluminescence.

Ejection of oxygen radicals into environment is one of the earliest responses of the immune system cells to an external stimulus. Since the discovery of the activation of oxidative metabolism in phagocytic leucocytes and their simultaneous chemiluminescence (CL) (Allen, Loose, 1976) it has been established that the oxygen radicals form enzymatically on a plasma membrane in the presence of oxygen and an energetic substrate. As external stimuli for cell activation, various soluble and insoluble substances have been used. Activated leucocytes, neutrophils, macrophages, thymocytes as well as T and B lymphocytes have been shown to be producers of oxygen radicals by this mechanism (Владимиров, Шестернев, 1989).

It has been suggested recently that small doses of irradiation may intensify the functional activity of immunocompetent cells (Ярилин, 1988). Accordingly, it has been shown that the formation of oxygen radicals in phagocytic macrophages increased after irradiation (Benichou et al., 1986; Gallin, Green, 1987). Evidently it reflected not as much the specific character of macrophages as the character of the generative system of oxygen radicals, and the stimulation of functional activity by irradiation may occur not only in macrophages but in other populations of the immune system cells as well.

The aim of the present study was to investigate the effect of irradiation on the *in vitro* production of oxygen radicals in the cells of central lymphoid organs of the chicken immune system, thymus and bursa of Fabricius. The CL of the cells activated by their adhesion to glass or nylon as well as the effect of irradiation on cell activation was studied.

MATERIALS AND METHODS

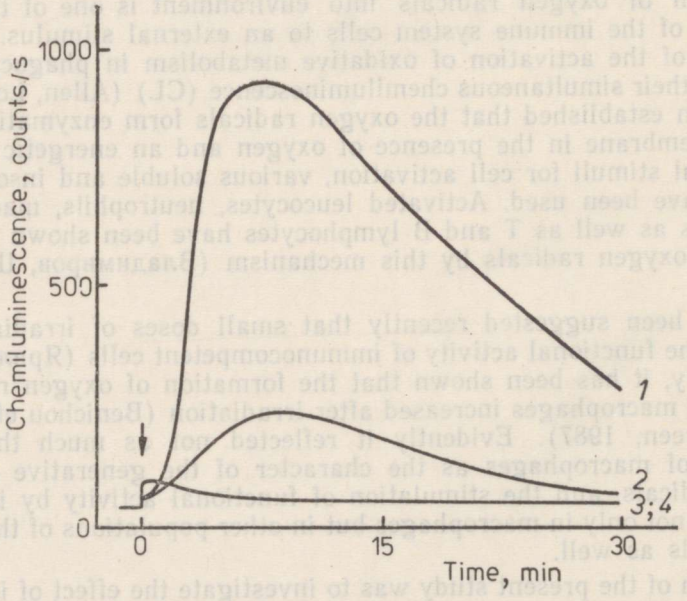
Cells were isolated from the thymus and the bursa of Fabricius of one-month-old broiler-chickens. After purification the cells were washed in Hanks' balanced salt solution (HBSS) and resuspended in HBSS with (concentration up to 6 mM) or without glucose. The suspension of the cells was irradiated *in vitro* with ^{60}Co γ-radiation. Doses from 1 to 10 Gy (dose rate 2.1 Gy/min) were used.

The CL was measured at 37 °C on a standard apparatus of the USSR origin complemented with a photon counter and a photomultiplier. CL was induced by adhesion of the cells to glass or nylon. The adhesion was effected by free sedimentation of the cells in 5-cm-diameter Petri dishes, the bottoms of which were covered or not covered with a nylon filter. Luminescence was registered from the upper side of the suspension (2×10^7 cells and 50 μM luminol). The material had been adapted to darkness before measurement. The results were expressed in counts per second (counts/s) and evaluated statistically.

RESULTS

The intensity of luminescence in the course of cell sedimentation in Petri dishes reached its maximum within 5...7 min, after which it began to decrease (Figure). It was possible to record CL during 30... 40 min after the thymocytes had been transferred to the measuring dishes. The sedimentation of the cells ended at about the same time.

Nylon increased the amplitude of CL several times and appeared to be a stronger activator of thymocytes than glass. However, the dependence of the CL intensity of the thymocyte suspension upon the incubation time in measuring dishes was similar for both materials. Practically no CL developed in the cell suspension of the bursa of Fabricius.



Luminol-dependent chemiluminescence of thymus (*1*, *2*) and bursa of Fabricius (*3*, *4*) cells adhering to glass (*2*, *4*) or nylon (*1*, *3*). The arrow marks the transfer of the cells into measuring dishes.

Dependence of the amplitude of luminol-dependent chemiluminescence of thymocytes on the doses of irradiation (per cent of control)*

Post-irradiation time, h	Radiation dose, Gy				
	0	1	2	5	10
3	100±14 (8)**	153±16 (8)	170±15 (5)	172±22 (6)	108±13 (6)
24	100±18 (8)	149±21 (6)	208±32 (4)	174±26 (6)	147±19 (6)

* control — nonirradiated thymocytes 3 h (852±119) and 24 h (629±112) after isolation, counts/s;
** number of measurements.

In order to elicit the nature of the appearance of CL, we studied the effect of glucose, scavengers of oxygen radicals and acetylsalicylic acid on the CL of thymocytes at their adhesion to nylon. Over 4-hour starving of thymocytes in a medium without glucose brought about an irreversible loss of the CL response. In the presence of 6 mM glucose in the medium, the cells preserved their ability of CL at least during 48 hours after isolation. Addition of 1 mM sodium azide (scavenger of singlet oxygen), 0.05% dimethylsulphoxide (scavenger of hydroxyl radical) or 0.4 mg/ml acetylsalicylic acid (inhibitor of prostaglandin formation) to the suspension of thymocytes led to the decrease in the CL amplitude by 32.6, 57.8, and 37.5 per cent of the control, respectively.

Gamma-irradiation of thymocytes modified the response of the cells to the contact with nylon (Table). At relatively small doses of irradiation (1 ... 5 Gy) stimulation of thymocytes, manifested as an increase in CL amplitude, was observed. At the doses of irradiation over 5 Gy, the effect decreased, while at 10 Gy there were no differences between the CL intensity of intact and irradiated thymocytes. The increase of CL in irradiated thymocytes was not connected with lipid peroxidation. This conclusion can be drawn from the lack of induction of lipid peroxidation which was tested with thiobarbituric acid as well as with the phenyl-2-naphthylamine fluorescent probe. Also, there was no CL in the suspension of irradiated cells of the bursa of Fabricius either at the adhesion to glass or to nylon.

DISCUSSION

Our results confirm the earlier observations (Allen, Loose, 1976; Ginsburg et al., 1985; Wymann et al., 1987) that the development of CL is characteristic of the activation of some types of immune system cells. The basis of the phenomenon is the formation of oxygen radicals in the cells.

The first step in the stimulation of the enzymatic generation of reactive oxygen is the interaction of cells with alien molecules and particles (Владимиров, Шестернев, 1989). Evidently the enzymatic generation of oxygen radicals could be the main reason in the genesis of CL in our experiments as well. The decrease of CL when scavengers of oxygen radicals were used and the obligatory presence of glucose for the CL response to occur serve as confirmation of this opinion.

A certain degree of CL in thymocytes must have been caused by the products of arachidonic acid metabolism as can be judged by the effect of acetylsalicylic acid on their CL. The participation of arachidonic acid intermediates in the development of CL in activated thymocytes has been demonstrated earlier (Hume et al., 1981).

It was unexpected to us that the cells of the bursa of Fabricius — an organ where the lymphocytes of B-system immunity mature — failed to respond to activation with generating oxygen radicals. Since CL definitely appears at the activation of mature B-lymphocytes (Kapp et al., 1986), either the system of the formation of oxygen radicals in the precursors of B-lymphocytes is underdeveloped, or the present method for activating the cells of the bursa of Fabricius is not an adequate stimulus for the formation of oxygen radicals. In any case, the lack of CL at the adhesion of the bursa of Fabricius cells could be used in search for markers for B-lymphocyte precursors.

At the activation of irradiated thymocytes we observed an increase in the CL amplitude (Table) which points to a more intensive generation of oxygen radicals in the cells affected by irradiation than in the intact ones. Recently, similar results have been obtained in an *in vitro* investigation of the reactive oxygen formation in irradiated macrophages (Benichou et al., 1986). The similarity suggests that the stimulation of thymocytes and macrophages by irradiation may occur on the level of non-specific receptors which participate in the activation of the generative system of oxygen radicals. It cannot be excluded, however, that the increase in the production of oxygen radicals at relatively small doses of irradiation is a specific property of those cells which in activated form may turn into efficient producers of oxygen radicals.

The generation of oxygen radicals by some cell populations of the immune system may be important for the protective functions of organisms but chronic activation of these cells may bring about a superfluous and stable formation of oxygen radicals that may induce inflammatory processes as well as damage to tissues (Halliwell, 1984). The relatively long stimulative effect of gamma-irradiation on thymocytes allows us to suppose that these cells belong to one of the main mediators of the irradiative effect in living organisms.

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AKTIIVSE HAPNIKU VORMIDE TEKE INTAKTSETES JA KIIRITATUD TÜMOTSÜÜTIDES

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Kasutades kemoluminestsents(KL)meetodit on uuritud kiiritatud ja kiiritamata tümotsüütide ning Fabriciuse pauna (bursa) rakkude aktiveerumist nende adhesioonil nailonile. On näidatud, et tümotsüütide adhesioonil nailonile tekib luminoolist sõltuv KL. KL-i inhibeerumine aktiivse hapniku neelajate toimel viitab hapniku radikaalide produtseerimisele rakkudes. Tümotsüütide gammakiiritamine suhteliselt väikeste doosidega põhjustab KL-i intensiivsuse suurenemise. Fabriciuse pauna rakkude puhul KL-i teket ei täheldatud.

ОБРАЗОВАНИЕ АКТИВНЫХ ФОРМ КИСЛОРОДА В ИНТАКТНЫХ И ОБЛУЧЕННЫХ ТИМОЦИТАХ

Тоомас КЕЭП, Ээзи ВАРЬЕНД, Тийа МЯНД

Методом хемилюминесценции (ХЛ) исследована активация интактных и облученных клеток тимуса и фабрициевой сумки цыплят при их адгезии на капрон. Показано, что при адгезии на капрон тимоцитов возникает люминолзависимая ХЛ. Подавляемая перехватчиками активных форм кислорода (АФК) ХЛ, требующая присутствия глюкозы, указывает на активацию генерации АФК в тимоцитах. Гамма-облучение тимоцитов относительно низкими дозами вызывает увеличение интенсивности ХЛ, что свидетельствует о повышении эффективности АФК-продуцирующей системы. В тех же условиях в клетках фабрициевой сумки ХЛ не обнаружено.