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Intercellular Communication and Cell Cooperation in Growth Control of T-Lymphocytes*

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Abstract. T-lymphocyte stimulation is strictly dependent on cell cooperation. Cell contact dependent cell cooperation has been described as well as cooperation by soluble mediators, the lymphokines. We here present a unifying hypothesis proposing that both types of cell cooperation are performed by the same mediators.

Key words: Lymphocyte stimulation – Intercellular communication – Lymphokines

Morphogenetic and functional organization of multicellular organisms involves long- and short range cellular interactions. The fundamental role of cell contact is generally accepted; however, the mechanisms underlying "contact cooperation" are still unknown. Control of lymphoid cell proliferation has been shown to be dependent on cellular interactions, including activation by mitogens which bind to and activate the responding cell. Cellular interactions are 1) essential for proliferation and 2) can control the response of immune cells by positive and negative signals. Although our understanding of these processes is still very limited, studies on immune cell interactions seem to be the most advanced compared with other model systems. The cell membrane as the central site of cell cooperation has been mostly studied in lymphocytes and the modern concepts of receptors, the fluid membrane model, lateral mobility of membrane components and their interaction with cytoskeleton elements has mainly been developed studying lymphocyte membranes.

One line of our work has been focused on cell contact mediated cell interactions, this field being less established compared to studies on soluble mediators, the lymphokines. In the first section of this paper, we will give a survey on contact interactions between immune cells, then develop a hypothesis

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which may unify the cell contact concept with the long distance concept of cell interactions, and finally we will give a very short summary of the current state of lymphokine knowledge.

Lymphocyte triggering agents, such as antigens or mitogens, bind to the surfaces of responding cells, but they also interact with macrophages and related cells, so-called accessory cells. Using mitogens as stimulants cell membrane alterations are among the first reactions of lymphocytes: receptors aggregate, form patches and eventually caps, the fluidity of the membrane is increased, membrane-bound enzymes and transport mechanisms are activated.

In addition, lymphocytes form contacts with each other and also with accessory cells (Peters 1972, 1973, 1974; Peters et al. 1981; Frost et al. 1973; Werdelin et al. 1974; Nielsen et al. 1974; Peters and Schimmelpfeng 1978, 1979a, b). Our studies using time-lapse cinematography have revealed that lymphocytes are highly motile and in the early phase of stimulation form small or large clusters which contain at least one accessory cell. These clusters are extremely dynamic structures which are permanently entered by new-coming lymphocytes and left by others. If cell clustering is prevented, growth stimulation of lymphocytes is completely abolished, although they have bound the mitogen at the surfaces (Peters 1972). Studying the phenomenon of cell cooperation, Hülser and Peters (1971a, b, 1972) have found that among mitogen stimulated cells low-resistance junctions are formed, a result which could be confirmed by others in demonstrating dye exchange between agglutinated lymphocytes (Sellin et al. 1974; Borm et al. 1974). In a continuous measurement we could show that after attachment of the mitogen to the membrane junctions are formed within minutes (Hülser and Peters 1971a, b, 1972). These measurements have also been confirmed by others using microelectrode measurements (Oliveira-Castro et al. 1973; Gaziri et al. 1975). Also, junctions between macrophages have been demonstrated (Kanno et al. 1980).

On the ultrastructural level, some groups demonstrated junctional structures between macrophages and lymphocytes as well as between stimulated lymphocytes (Gaziri et al. 1975; Schoenberg et al. 1963; McIntyre et al. 1976; Siebert 1979; Kapsenberg and Leene 1979). So far there is no agreement on the classification of the junctions which are designated as septate junctions (McIntyre et al. 1976; Siebert 1979) or as gap junctions class B (Kapsenberg and Leene 1979).

As a third attempt of demonstrating junctions between cells the phenomenon of "metabolic cooperation" has been used. Cells carrying certain genetic defects should transiently be cured by contact with wild type cells which can transfer the missing metabolite by junctional communication. However, so far it was not possible to demonstrate a transfer of the missing metabolite in lymphoid cells (Cox et al. 1976; DeBruyn and Oei 1973). Therefore, on the basis of these results, the phenomenon of metabolic cooperation in lymphocytes remains unresolved; animal models or long-term cultured lymphocytes may permit more defined experiments.

Much experimental work has been spent to prove a specific information transfer by DNA or RNA between lymphocytes, which would permit a rapid horizontal expansion of specific information. However, an experimental proof

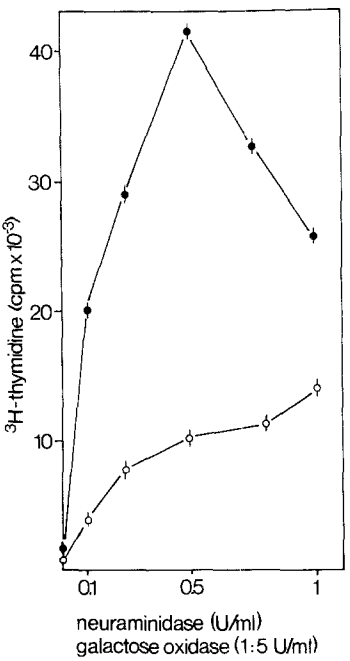

Fig. 1. Indirect lymphocyte stimulation by mitogen-pulsed macrophages. 3×10^4 mouse peritoneal macrophages were allowed to adhere to either the flat bottom of a microtiter plate or to a plastic cover slip. They were then treated with various concentrations of the lymphocyte mitogen neuraminidase plus galactose oxidase (abscissa), washed free of the enzymes, and traces of the mitogenic galactose oxidase were competed for by added D-galactose. 6×10^5 purified mouse spleen lymphocytes were given either to the macrophage containing microwells or into empty wells which thereafter received the small coverslips containing the mitogen-pulsed macrophages. Whereas in the former set-up the lymphocytes got direct contact with the pulsed macrophages, in the latter set-up macrophages on the coverslip remained without physical contact with the settled lymphocytes. In this case they could communicate with lymphocytes only by soluble factors via the culture medium. The resulting stimulation of lymphocytes was determined 2 days later by tritium thymidine incorporation (ordinate). It is shown in the figure that already at low mitogen concentrations lymphocytes became stimulated by cell contact with the mitogen-pulsed macrophages (●———●), whereas in the absence of cell contact between macrophages and lymphocytes (○———○) only a marginal stimulation can be observed. At higher mitogen concentrations which finally are cytotoxic increasing amounts of soluble mediators are released from the macrophages. All cultures received 1% polyethylene glycol which renders lymphocytes more susceptible for the macrophage-derived signals (Peters and Schimmelpfeng 1978)

that nucleic acids are transfered from cell to cell is still missing for lymphocytes.

For contrast, it has been shown that lymphocyte subpopulations can control each other by the exchange of soluble mediators, "lymphokines". These mediators can act on a long range. However, we have found that during the contact interaction between accessory cells and lymphocytes soluble mediators are not detectable in many cases. For this situation two possible explanations may be discussed: (1) Lymphocyte mitogenesis as mediated by cell contact with accessory cell depends on a mechanism completely different from the exchange

of lymphokines; lymphokines would then be classified as modulators rather than main triggers of the lymphocyte response. (2) These molecules are in some cases exchanged directly from cell to cell without entering the extracellular fluid. In the experiment shown in Fig. 1 evidence is given to support this latter model: in a mitogenic cell cooperation system, where lymphocytes were stimulated by mitogen-pulsed accessory cells various concentrations of the mitogen (in this case a combination of neuraminidase and galactose oxidase) were used. At low mitogen concentrations inducing significant lymphocyte stimulation very little stimulatory lymphokine could be detected. At higher mitogen concentrations increasing amounts of stimulatory material was detected outside the cells, but these concentrations obviously impaired cell viability as judged by phase contrast observation. We therefore conclude that it is possible that during optimal lymphocyte stimulation mediators may be exchanged on a short range. Only under extreme situations will they appear in the extracellular fluid.

When lymphokine containing supernatants were analysed, several mediators have been found (Paetkau 1981). Two of them have been designated as interleukins (Aarden et al. 1979). Interleukin 1 is released by macrophages and related cells after activation. It seems to be a single polypeptide chain of a molecular weight of 12–16 K Dalton (Mizel and Mizel 1981). It is released after strong activation of the macrophages or macrophage lines. After lysis of the macrophages more Interleukin 1 activity is found in the supernatant. However, lysis of non-stimulated macrophages does not result in Interleukin 1 activity. This again suggests that (as in the experiment described above) Interleukin 1 is the limiting factor for induction of lymphocyte proliferation.

Interleukin 2 is released by T cells. It seems to be a single polypeptide chain of about 15 (human) or 30 (mouse) K Dalton (Gillis 1982). T-cells of several

Table 1. Interleukin 1 response of an Interleukin 2 dependent long term T-cell line

Experiment Interleukin 1			Experiment Interleukin 2	
Percent P338 D1 CM	−Con A cpm ^{3}H-thymidine	+Con A cpm ^{3}H-thymidine	Percent EL4 CM	−Con A cpm ^{3}H-thymidine
25	60	9,930	6	6,430
12.5	45	14,330	3	8,622
6	120	13,510	1.5	9,933
3	220	6,590	0.6	6,310
1.5	110	3,630	0.3	4,510
Medium control	50	1,100		

The proliferation of a helper T cell line originally known to be Interleukin 2-dependent is measured on day 3 in microtiter cultures at a cell concentration of 2×10^4 cells/well by ^{3}H-thymidine incorporation. To the cultures El 4 cell conditioned medium (CM) (Schmidt et al. 1982) as a source of Interleukin 2, P388 D1 cell CM as a source of Interleukin 1, Concanavalin A (Con A) at a concentration of 10 μg/ml were added. The results are expressed in cpm of incorporated ^{3}H-thymidine. This T cell line responds to Interleukin 1 + Con A in an Interleukin 1 dose dependent fashion. This cell line could be used for studying mechanisms underlying mitogen stimulation. It was produced and kindly provided by Dr. Frances I. Smith (Institute for Genetics, Cologne).

subclasses are able to grow in the presence of Interleukin 2 after prior activation by antigens or mitogens (Nabel et al. 1981). Interleukin 2 seems specifically to act on T-cells which carry a specific receptor. Experiments performed to analyse the mechanism of Interleukin 2 release have revealed that

1) it is mainly produced by helper T-cells of the phenotype: Lyt 1^+, 2^-,

2) accessory cells are needed for the production of Interleukin 2,

3) the accessory cells can be replaced by Interleukin 1 (Larson and Coutinho 1979; Larson et al. 1980).

In the experiment shown in Table 1 an Interleukin 2 dependent T-helper cell line was used to measure the effect of Interleukin 1, demonstrating that long term lines can be used to study the mechanism of mitogen stimulation.

In contrast to Interleukin 2, Interleukin 1 itself seems to be a molecule acting not only on T-lymphocytes but even on non-lymphoid cells such as fibroblasts (Schmidt et al. 1982). This mediator may be involved in many different cellular systems and may also be produced by non macrophage cells. In future, a more extended use of cloned cell lines should help to further dissect cell-cell interaction between accessory cells and responding lymphoid cells as well as between subsets of lymphocytes.

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