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THE ROLE OF THYMIC ACCESSORY CELLS IN CYTOKINE PRODUCTION AND IN THE INTRA-THYMIC T CELL DIFFERENTIATION PATHWAY

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Intrathymic T lymphocyte differentiation proceeds from complex interactions between prothymocytes of bone marrow origin and cells of the thymic stroma, epithelial cells and "accessory" cells (macrophages and/or interdigitating cells). The present paper describes the role of the accessory cell compartment in this intrathymic process. Accessory cells produce factors which are involved in thymocyte proliferation (interleukin 1, prostaglandins, deoxynucleosides). Cell-cell interaction between "accessory" cells and thymocytes is required for the regulation of interleukin production. Prothymocytes, the precursors of all thymocyte subsets, need the accessory cell compartment for their IL 2 dependent proliferation and their differentiation. Accessory cells of the thymic stroma may be involved in the intrathymic selection process at the prothymocyte level.

The role of the thymus is to transform immature prothymocytes from the bone marrow, into mature T cells able to colonize the peripheral lymphoid organs. While entering the thymus, thymocyte precursors are submitted to multiple signals which lead to their proliferation and to the acquisition of membrane differentiation antigens, T cell receptor expression and T cell functions. Interaction of prothymocytes with cells of the thymic stroma, which are either epithelial or accessory cells (macrophages and interdigitating cells), is a prerequisit for this maturation process. Production of factors in the thymic micro environment during cell to cell interaction is involved in prothymocyte proliferation, selection and maturation. Different types of factors have been shown to be produced in the thymus by different types of cells. Thymic hormones are produced by the epithelial compartment of the stroma (Bach, 1983; Bach et al., 1984). Interleukin 2 (IL2) interferon γ (IFNγ) may be produced by intrathymic T lymphocytes (Ceredig et al., 1982; Reem et al., 1983). Colony stimulating factors (CSF) such as granulocyte-macrophage CSF (GM-CSF) or interleukin 3 (IL3) (Ceredig, 1986) may also be produced by early T cells, while thymic fibroblasts produce CSF-1 (macrophage-CSF) (Papiernik & Savino, unpublished results). Thymic accessory cells are responsible for the production of interleukin 1 (IL1) and prostaglandins (PGs) (Papiernik & Homo-Delarche, 1983; 1987). The present report will focus on the production of cytokines by cells of the thymus and on the interrelationships between lymphocytes and bone marrow derived accessory cells.

Intrathymic interleukin production – To study the role of accessory cells of the thymic stroma in cytokine production and their relationship with thymocytes, we developed a technic to isolate them in vitro (Papiernik et al., 1983). Briefly, primary cultures are established by seeding small fragments of thymic tissue into plastic flasks. During the first week of culture, all thymic cells are represented, including thymic stromal cells and thymocytes. By the end of the first week, lymphocytes have disappeared and monolayers of thymic stromal cells are established. These primary culture monolayers contain mainly thymic accessory cells (thymic macrophages and cells with a dendritic shape) as well as some epithelial cells and fibroblasts (Papiernik & Nabarra, 1981). On the surface of these monolayers, round cells with a membrane having a hairy aspect proliferate. They are nonadherent and are released into the culture medium from which they can be recovered and replated in secondary cultures where they become adherent. These cells are called phagocytic cells of the thymic reticulum (P-TR). They are I-A$^+$, Mac 1+ cells with a dendritic shape. They display a close relationship with thymocytes and stimulate the proliferation of thymocytes in syngeneic coculture (El Rouby et al., 1985; Papiernik et al., 1983; Papiernik, & El Rouby, in press). The thymic microenvironment was analyzed by measuring the production of different factors, mainly PG, IL-1 and IFNγ, in primary cultures. To analyze more precisely the role of accessory cells, P-TR function and the production of PG and IL-1 was studied in secondary cultures.

Production of IFN-γ, PGs and IL1 in thymic stromal cell cultutes – Interferons are known to be immunoregulatory molecules with either suppressive or enhancing potentialities (Sonnenfeld, 1980). It has also been shown that IFN-γ is able to modulate MHC antigens on the surface of various types of cells (Steeg et al., 1980; Wong et al., 1984) and to modulate IL-1 release by accessory cells (Boraschi et al., 1984a; Durum et al., 1984). We were interested in the local production of IFN-γ, as we found that the expression of class II MHC antigens on the surface of P-TR recovered in the primary cultures was conversely proportional to the age of the primary culture. As thymocytes are present during the first week of primary cultures and are known to be able to produce IFN-γ (Reem et al., 1983), we hypothesized that local secretion of IFN-γ may be responsible for this variability in I-A expression. Indeed, we find that early primary culture supernatants have IFN activity. The addition of anti-IFN-γ antibody to primary cultures inhibits I-A expression on newly produced P-TR, showing that IFN-γ production is indeed responsible for this effect (Papiernik et al., 1986).

PGs are also produced in the primary cultures of thymic stroma (Papiernik & Homo-Delarche, 1987). PGE2, 6-keto PGF1 α and PGF2 α were found, and their production increased while IFN-γ production disappeared.

IL 1 like activity was also detected in the primary culture supernatant, using the comitogenic assay on thymocytes. IL1 like activity can be detected only if PGs are removed from the supernatant by dialysis. Indeed, IL1 and PGs have an antagonistic effect on the proliferation of thymocytes (Papiernik & Homo-Delarche, 1983). Furthermore, PGs have been shown to inhibit IL1 production (Boraschi et al., 1984b; 1985) and we demonstrated that the production of IL1 is enhanced when PG production is blocked by indomethacin. However, even in the presence of indomethacin, IL1 like production decreased with time in the primary cultures (Papiernik & Homo-Delarche, 1987). It has been shown that INF-γ and IFN-α are natural up-regulators of IL1 production. Expression of class II antigens are also up regulated by IFN-γ. It seems then that Ia expression and IL1 release are coordinately regulated (Durum et al., 1984; Durum et al., 1985). And as Ia expression in macrophage membrane controls IL1 release, it is clear that thymic macrophages and thymocytes are tightly linked through IFN-γ, PGs and IL1 secretion. IL1 secretion is a necessary signal for the release of the T cell growth factor interleukin 2 (IL2), and for the IL2 response of T cells. Figs. 1 and 2 summarize these interrelationships between the production of INF-γ, PGs and IL1 by thymic accessory cells and thymocytes.

Growth factors for accessory cells and thymocytes – The only lymphocytes which express receptors for IL2 in the thymus are the thymocyte precursors which do not express Lyt2 or L3T4 differentiation antigens (Raulet, 1985). These double negative cells (DN cells) are able to give rise to all thymocyte subsets when injected in vivo (Mathieson & Fowlkes, 1984) or associated in organ culture with thymic stroma (Kingston et al., 1985).

Despite the fact that 50% of DN thymic cells express IL2 receptors, it was shown by several groups that IL2 in vitro induces very poor proliferation of DN cell populations (Ceredig et

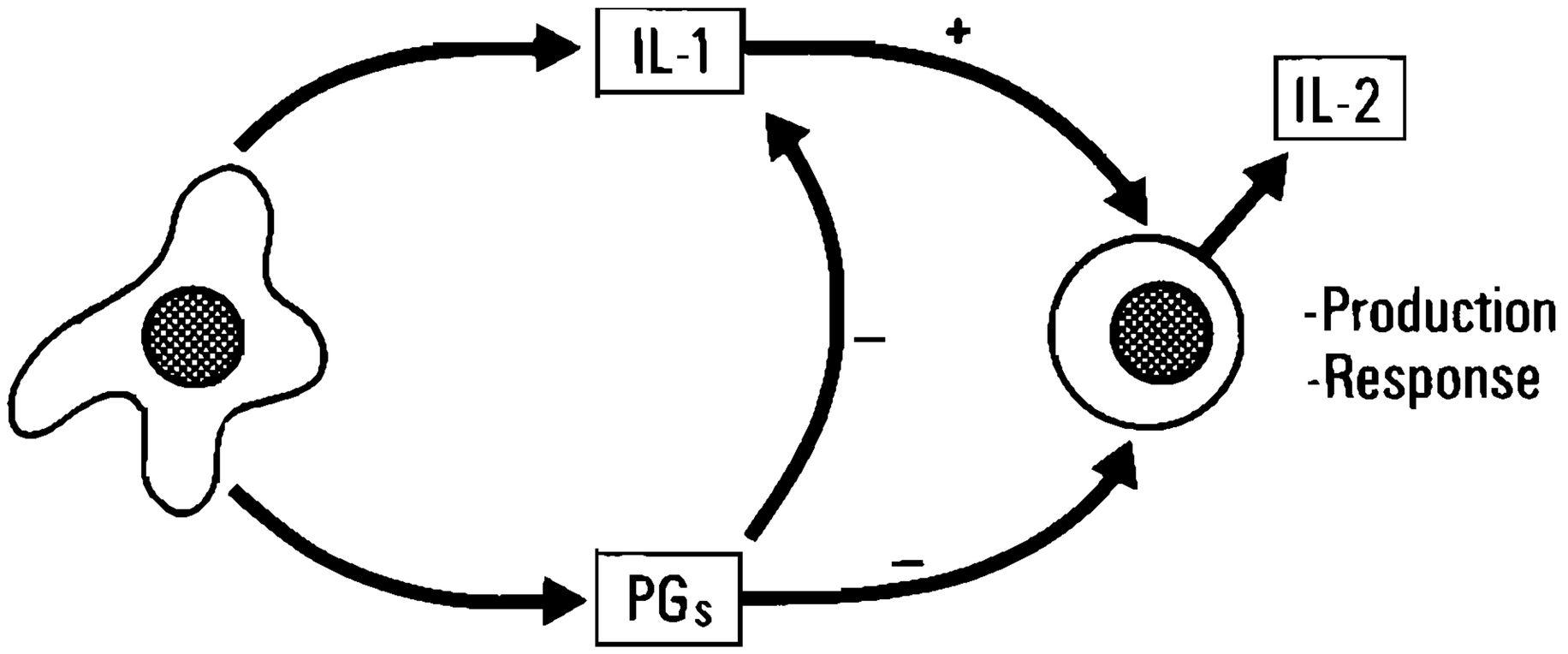

Fig. 1 : Phagocytic cells of thimic reticulum secrete both IL-1 and PGS which have an antagonistic effect on thymocyte proliferation by acting on both IL-2 secretion and action. PGS are also able to down-regulate IL-1 production.

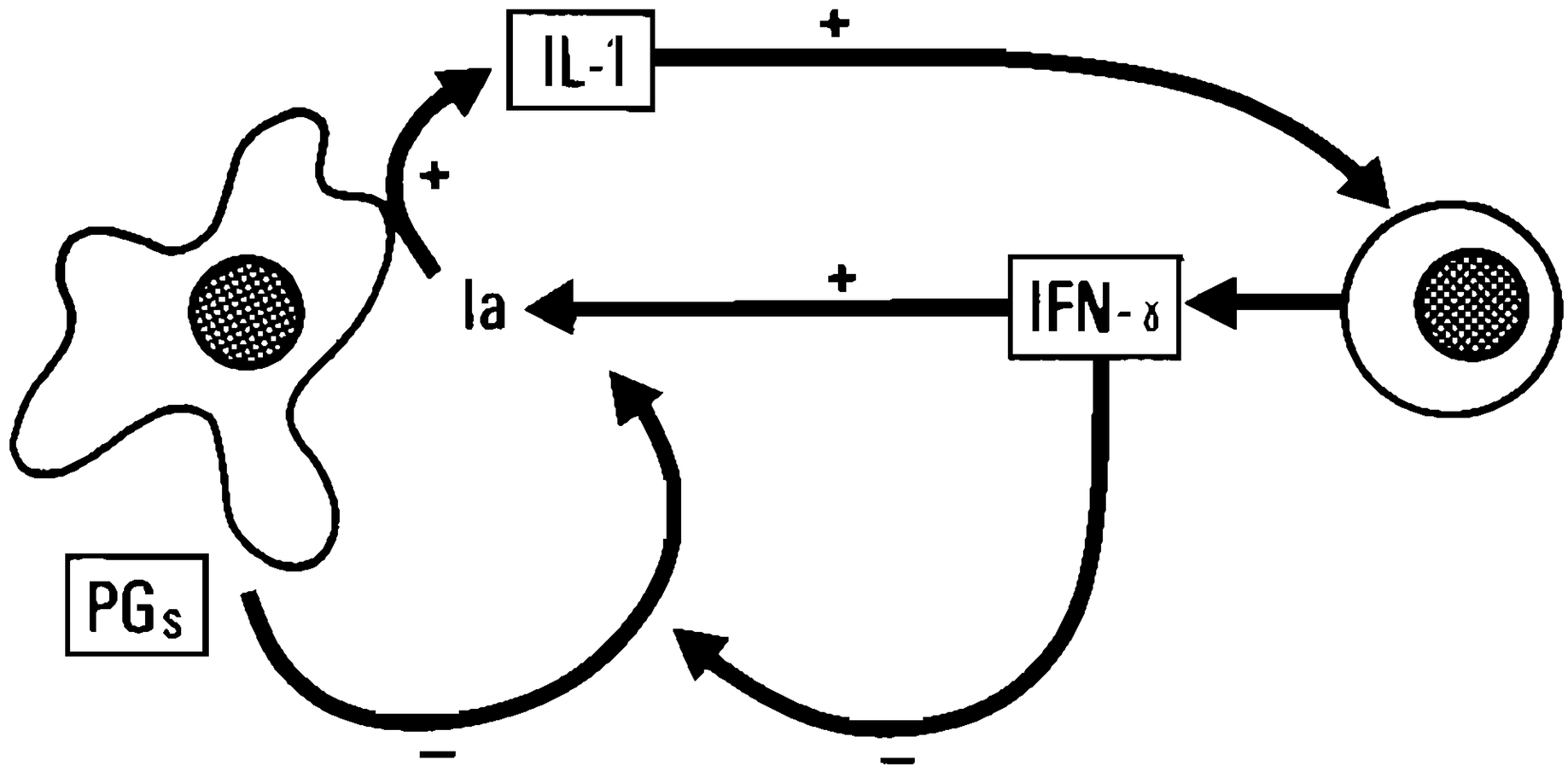

Fig. 2: Thymocytes may also interfere with IL-1 and PGS production mainly by the production on IFN-γ which act by down regulating PGS production and by up-regulating Ia antigen expression on phagocytic cells of the thymic reticulum. Ia expression controls the IL-1 release by macrophages.

al., 1985; Raulet, 1985; Palacios & Von Boehmer, 1986; Ceredig, 1986). Activators of protein kinase C such as phorbol myristate acetate may induce the proliferation of prothymocyte precursors and replace the in vivo signal(s). We have recently shown that thymic accessory cells may provide the physiologic signal for DN cell proliferation (Papiernik et al., 1987). Indeed, when DN thymocytes were associated with P-TR, IL2 was able to induce DN cell proliferation and to maintain the generation of more mature thymocytes, expressing differentiation antigens L3T4 and/or Lyt 2 (Papiernik et al., 1987) (Fig 3). Unpublished data seems to indicate that IL1 may partly replace thymic accessory cells, and may be the main intermediary factor which mediates this effect.

Thymic accessory cells may also be dependent upon thymocytes and pro-thymocytes for their growth.

We have recently shown that DN thymic cells not only contain thymocyte precursors but also hemopoietic precursors (Papiernik et al., in press). These precursors may differentiate into macrophages in the presence of GM-CSF or multi-CSF (or IL3). Such CSF have been shown to be produced by activated T cells but also by stimulated DN thymic cells in vitro (Ceredig, 1986). Both types of precursors are thus tightly linked for growth induction. Furthermore, phagocytic cells of the thymic reticulum (P-TR) have been shown to express IL2-R in vitro and to proliferate in the presence of IL2 (Rocha et al., 1987). These results underline the complex inter-relationship between

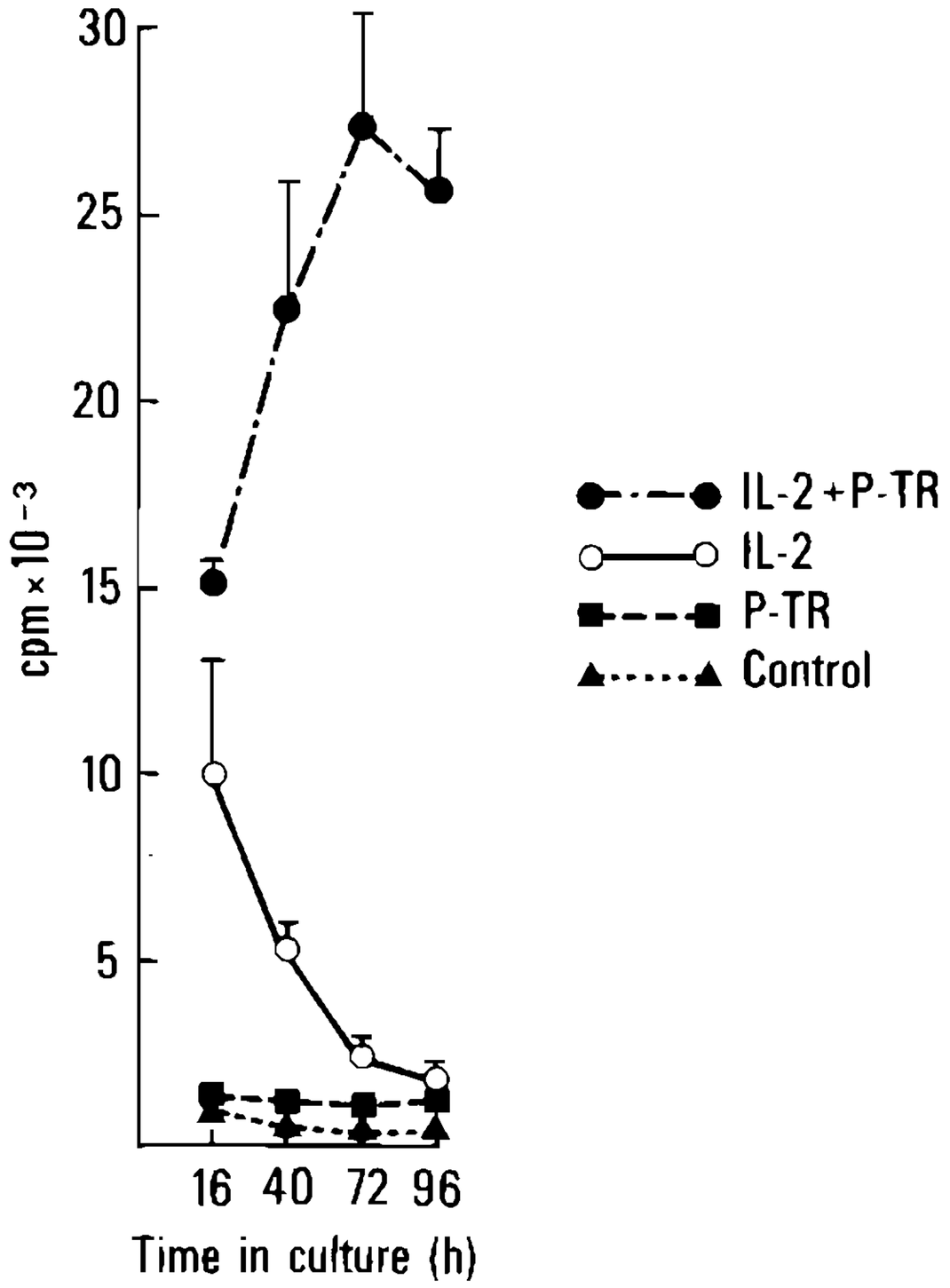

Fig. 3: L3T4⁻ Lyt2⁻ prothymocytes poorly respond to IL-2. Interaction of prothymocytes with phagocytic cells of thymic reticulum in vitro (P-TR) induce the IL-2 dependent proliferation of prothymocytes.

the different types of cells and their growth factors.

Thymocyte/Thymic accessory cell interactions – When thymocytes are mixed in vitro with thymic accessory cells, thymocytes are

induced to proliferate (Papiernik et al., 1983, Papiernik & El Rouby, in press). This syngeneic reaction is a self sustained phenomenon where all the factors needed for thymocyte proliferation are locally produced. The results described above show that all growth factors may be intrathymically produced. This close relationship between thymocytes and thymic accessory cells is visualized by the formation of rosettes between the two types of cells (El Rouby et al., 1985). Thymocytes which bind to P-TR in vitro are mainly of the cortical type, Lyt2+ L3T4+ (90%), whith a small proportion of Lyt2- L3 T4+ mature helper type T cells (3%) of Lyt2- L3T4- precursors (6%), and very few mature Lyt2+ L3T4- cytotoxic type T cells (El Rouby & Papiernik, 1987). Then production of IL1 and PGs (by thymic accessory cells), production of IFNγ (by the Lyt2+ L3T4- thymocyte subpopulation) or IL 2 (by the Lyt2- L3T4+ thymocytes) is a likely phenomenon in this in vitro P-TR/thymocyte interaction.

The role of the cortical thymocytes which bind to P-TR is an interesting question. Indeed, most of the thymocytes which express the cortical phenotype are destined to death and will be destroyed intrathymically. At least part of the Lyt2+L3T4+ cells which are binding to P-TR are endocytized. And this may be the way by which cortical thymocytes are cleared from the thymic milieu. One interesting point is the production of deoxynucleosides during this process. We have shown (Penit & Papiernik, 1986) that P-TR are able to produce both deoxycytidine and thymidine, and that this production is greatly enhanced when thymocytes are added to P-TR. Degradation of apostotic thymocyte DNA by thymic accessory cells may thus be the mechanism of deoxycitidine and thymidine production. These deoxynucleosides can be reutilized by thymic blasts and their proliferation enhanced. Each thymocyte rosette in vitro may represent one functional unit of thymocyte/thymic accessory cell interaction, which leads to thymocyte proliferation. Binding of cortical and mature type thymocyte is inhibited by anti-Mac-1 antibody (El Rouby et al., 1985) which recognize the third receptor of complement. Mac-1, like LFA-1, belongs to a family of molecules which is involved in cell to cell interactions. Mac 1 also inhibits thymocyte proliferation during syngeneic mixed lymphocyte culture between P-TR and thymocytes, showing that thymocyte binding is a prerequisit for proliferation. When the cultures comprise all types of thymocytes, the majority of the cells which proliferate is of the Lyt2-L3T4+ phenotype and also needs MHC class II antigen recognition (Papiernik, & El Rouby, in press). P-TR/DN thymocyte interaction also leads to proliferation. In that case, proliferation is not a self-sustained phenomenon, as the IL2 producers are missing, and exogeneous IL2 must be added to the culture medium (Papiernik et al., 1987). Proliferation is linked to membrane antigen differentiation and the P-TR microenvironment is sufficient to maintain a constant small population of antigen positive cells with a short life span. DN cells also bind to P-TR (El Rouby & Papiernik, 1987) but antigen(s) involved in this binding are not known. DN cells which proliferate seem to be mainly included in the binding subpopulation (El Rouby & Papiernik, submitted) and proliferation is inhibited by anti-MHC class I antibodies (Papiernik et al., 1987). Fig. 4 summarizes the accessory cell/thymocyte interactions.

Conclusions: Thymocyte/Thymic accessory cell interaction, a functional unit for thymocyte selection and proliferation.

Bone marrow derived thymic acessory cells are made of both interdigitating cells located in the medulla and at the cortico-medullary junction, and of macrophages which are scattered throughout the thymus and concentrated in the cortico-medullary junction and along blood vessels. Then, if thymic accessory cells are a minor component of the stroma, they may be important partners of lymphocyte intra-thymic life: Thymic accessory cells may be the first stromal cell a prothymocyte encounters while entering the thymus through blood-vessels, and also the last stromal cell the mature cell may encounter when leaving the thymus for the periphery. And then thymic acessory cells may be part of the intra-thymic selection/maturation pathway which leads to the seeding of peripheral organs with mature T cells.

One of the first steps of this intrathymic maturation pathway may be the binding of DN pro-thymocytes to thymic accessory cells. Whether this DN binding is an active selection process or is a random phenomenon depending on the point of entry into the thymus is not known. The fact is that DN pro-thymocytes do not proliferate in the presence of IL2 alone, (which is intended to be their growth factor) although 50% of them have receptors for IL2. We have shown that interaction of DN prothymocytes with P-TR in vitro gives the necessary

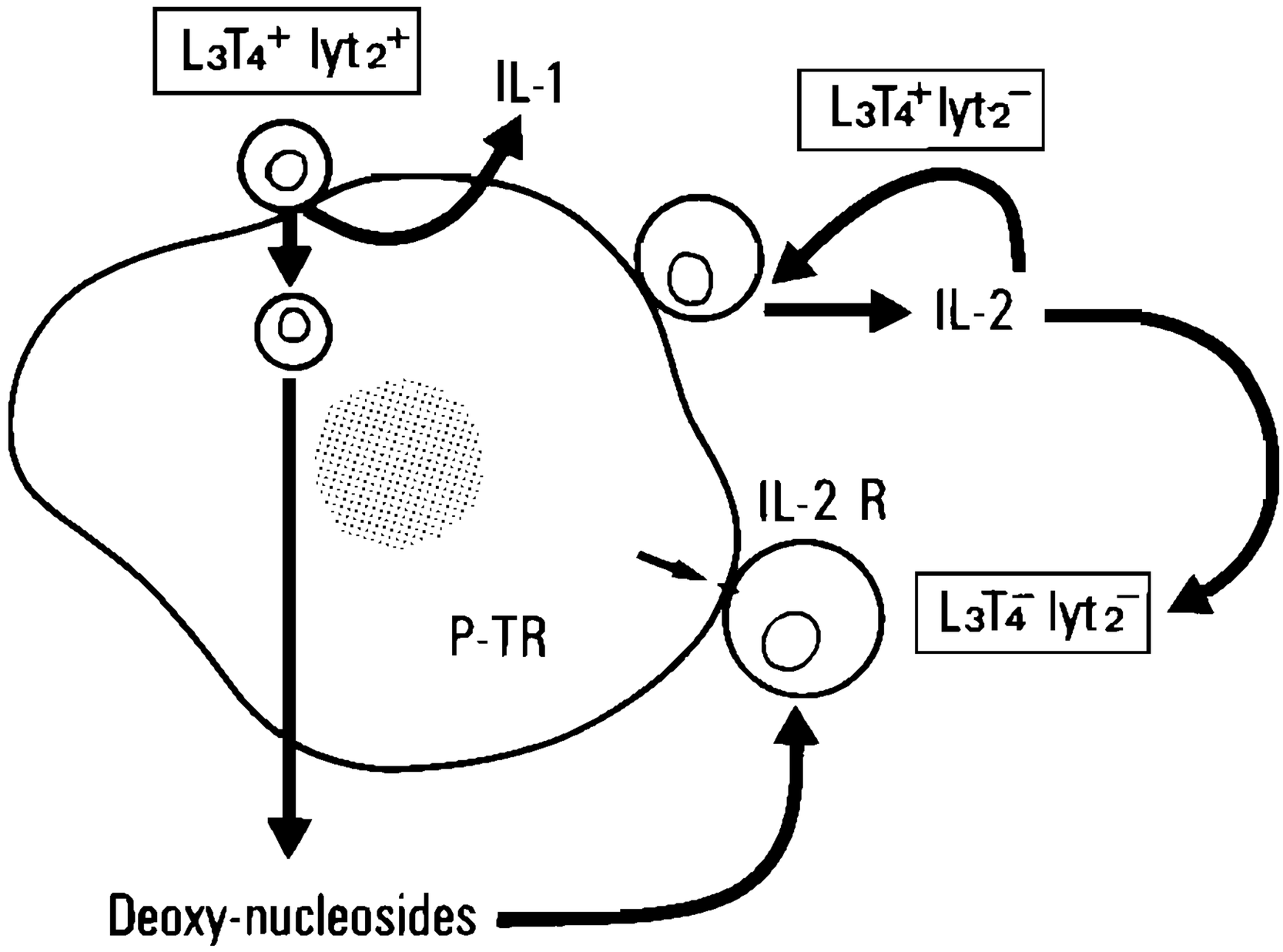

Fig. 4 Summary of the interactions between thymic accessory cells which were grown in vitro as phagocytic cells of the thymic reticulum (P-TR) and thymocytes. Different types of thymocytes may interfere with P-TR: L3T4$^+$ Lyt2$^+$ cortical type thymocytes may be endocytozed, their DNA is then degraded and the doxynucleosides reutilized by proliferating blasts. Thymocyte binding may also induce IL1 production which is needed for the IL2 production and action. IL-2 may be produced either by L3T4$^-$ Lyt2$^-$ prothymocytes but more likely by L3T4$^+$ Lyt2$^-$ mature thymocytes. IL-2 acts both on L3T4$^+$ Lyt2$^-$ thymocytes and on L3T4$^-$ Lyt2$^-$ prothymocytes. Interaction of prothymocytes with P-TR is a necessary signal for their IL-2 dependent proliferation. Production of IL-1 is needed for this effect. The effect of P-TR on the production of high affinity IL-2 R is also likely.

signals for the IL2 dependent proliferation of pro-thymocytes (Papiernik et al., 1987). Then part of DN thymocytes are selected for proliferation by interaction with thymic accessory cells. What is the mechanism of IL2 production? In vitro, DN cell/accessory cell interaction is not sufficient for IL2 production, and IL2 has to be added in order to induce DN cell proliferation. Several hypotheses may be suggested: 1. DN prothymocytes may produce insufficient IL2 in vitro; 2. DN cells do not produce IL2 during accessory cell interaction and another signal is needed provided by another type of stromal cells (epithelial cells ?); 3. DN thymocyte do not produce IL2 and a third partner is needed for IL2 production. This is illustrated in the rosette formation where both DN cells and IL2 producers (Lyt2-L3T4+ cells) are present. Interaction of these three partners may occur at the cortico-medullary junction or in the deep cortex where it is not unlikely that DN cells enter. We have documented above that IL1, PGs and deoxynucleosides are produced by thymic accessory cells, and are part of the factors which are needed for thymocyte proliferation. Interaction between thymocytes and accessory cells is a bi-way activation process which leads to thymocyte proliferation and selection at the DN cell level. It remains to determine whether the rearrangements of the T cell receptor genes, which is a major step in intra-thymic T cell maturation, is associated to the DN cell proliferation, and if complementary signals by other stromal cells are needed.

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