

SOLUBLE CD23 (FcεRII) AND
INTERLEUKIN 1 SYNERGISTICALLY INDUCE
EARLY HUMAN THYMOCYTE MATURATION

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During development, lymphoid stem cells migrate into the thymic rudiment where they differentiate into functionally mature T lymphocytes (1). Among human thymocytes, CD7⁺CD2⁻CD3⁻CD4⁻CD8⁻ (referred hereafter as CD7⁺) cells represent the earliest identifiable step (2-4). In vitro, these precursors were able to acquire mature T cell antigens (3, 4), but cellular interactions and cytokines necessary for this process are poorly understood. We have previously reported that enriched populations of B lymphocytes from blood produce factors (3, 5) that promote in vitro development of blood- and bone marrow-derived CD7⁺CD2⁻ precursors. Comparing the biochemical characteristics of this B cell-derived activity (3) to known B cell-derived molecules, we found its striking homology to soluble CD23 (sCD23; sFcεRII) (6, 7). Using recombinant sCD23 (rsCD23) (7), we then assayed the effect of this molecule alone or with rIL-1 and/or rIL-2 on purified CD7⁺ thymic precursors. Our results provide direct evidence that sCD23 and rIL-1 synergistically induce CD7⁺ prothymocytes maturation into CD2⁺CD3⁺TCR-α/β⁺CD4⁺ and/or CD8⁺ cells that respond to CD2 triggering and rIL-2.

Materials and Methods

mAb and Marker Analysis. Used in this study were OKT6(CD1), OKT11(CD2), OKT3(CD3), OKT4(CD4), OKT8(CD8), and OKM1(CD11b) (Ortho Pharmaceutical, Raritan, NJ); IOM2(CD14), IOB4(CD19), IOB6(CD23, IgG.K), and IOT14 (CD25) (Immunotech, Marseille, France); WT31 (recognizing CD3/TCR-α/β complex; Becton Dickinson & Co., Grenoble, France); RFT2 (CD7) (2); 6C2 (anti-TCR-γ) (8); and δTCS-1 (anti-TCR-δ; T Cell Sciences, Cambridge, MA). Fluorescein-conjugated anti-mouse F(ab)₂ fragments (Becton Dickinson & Co.) were then applied. Cell markers are evaluated using a FACStar (Becton Dickinson & Co.) or UV microscope (E. Leitz, Inc., Rueil, France).

Cells. Thymocytes were obtained by teasing thymic fragments. Prothymocytes were iso-

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lated by two-step adherence on plastic surfaces followed by two cycles of cytotoxic treatment with OKT11A, OKT3, OKT4, and OKT8, and subsequent incubation with newborn rabbit complement (3). The cells were then applied into flasks coated with RFT2 mAb (3, 5). Panned (CD7⁺) cells were then collected and centrifuged on Ficoll gradient to eliminate dead cells. The recovered cell numbers correspond to ~0.3% of unfractionated thymocytes.

Cell Cultures. Thymic isolated CD7⁺ cells were incubated 48 h in MacCoy's 5A medium containing 20% human AB serum and one or a combination of: rsCD23 (25 ng/ml) (7), rIL-1 (50 U/ml rIL-1 β), rIL-2 (100 U/ml) (rIL-1 β and rIL-2 were a kind gift from Glaxo, Geneva, Switzerland), IOB6 (CD23, IgG1.K), IOB4 (IgG1.K, 25 μ g/ml), and IOT14 (10 μ g/ml) (Immunotech). The cells were then washed and tested for their surface markers. In this assay, >88% (range, 88–98%) of cultured cells were recovered and viability exceeded 94%. We failed to detect cell proliferation during this period, regardless of culture conditions used (assayed by [³H]thymidine uptake, data not shown). For the proliferation assay, CD7⁺ cells were first incubated with various factors for 24 h (as above). They were then washed and cultured with anti-CD21+III mAb (1/400 ascite; a gift of A. Bernard, Institut Gustave Roussy, Villejuif, France) (9) and 100 U/ml rIL-2. [³H]Thymidine was incorporated on day 5, and radioactivity uptake was measured on day 6. These cells were also cultured in limiting dilutions (3). Briefly, CD7⁺ cells were incubated 48 h with various factors, washed, and cultured (0.1–100 cell/well) in 96-well microplates in the presence of CD21+III, rIL-2, and 10³ autologous irradiated (5,000 rad) CD2⁻ cells. Positive (>100 cells) vs. negative wells were scored from day 10, as described (3).

Results and Discussion

The surface markers of CD7⁺ cells were analyzed before and after incubation with either rsCD23, rIL-1, rIL-2, or various combinations of these molecules. Freshly isolated cells were mostly CD7⁺CD2⁻CD3⁻CD4⁻CD8⁻ (Table I), and did not express TCR- α/β or TCR- $\gamma\delta$. Upon incubation in culture medium alone, the phenotype of these precursors remained unchanged ($p < 0.001$), indicating that there was no surface antigen modulation. The data in Table I show that maturation of CD7⁺ precursors requires both rIL-1 and rsCD23, in as much as the expression of the maturation antigens (CD2, CD3, TCR- α/β , CD25, CD4, and/or CD8), was observed only in the cultures containing these two recombinant molecules ($p < 0.005$). This effect was completely suppressed by anti-CD23 mAb, but not by the addition of an isotype-matched unrelated mAb (anti-CD19, IgG1.K). The elimination of CD14⁺, MHC-II⁺, and/or LFA-3⁺ cells from CD7⁺ cell preparations did not significantly affect rsCD23 + rIL-1-derived responses (data not shown).

We next examined whether the acquisition of T cell differentiation antigens was accompanied by a functional maturation. To this end, CD7⁺ cells, preincubated as in Table I, were tested for their capacity to proliferate in response to a mitogenic pair of anti-CD2 (9) and rIL-2. As seen in Fig. 1, preincubation with either rIL-1, rIL-2, or rsCD23 did not induce CD2 responsiveness. By contrast, cells preincubated with both rsCD23 and rIL-1 showed a clear proliferative response. The addition of rIL-2 to rsCD23 + rIL-1 further increased the growth potential ($p < 0.01$). These data further confirm the absence of CD2 expression by freshly isolated CD7⁺ thymocytes.

To provide further evidence that prothymocyte maturation required sCD23, we treated CD7⁺ precursors for 48 h with increasing amounts of rsCD23 and constant rIL-1 and rIL-2 concentrations, and then we tested the proliferative responses to anti-CD2 and rIL-2, as well as CD2 and CD3 expression. Fig. 2 shows that the rsCD23 effect is dose dependent, with an optimum of 25–75 ng/ml.

TABLE I
Surface Labeling of Human Thymus-derived CD7+ Cells Before and After Incubation with Various Factors

Cells	Percent of positive cells												
	CD1	CD2	CD3	CD4	CD8	CD4, 8	CD7	CD14	CD19	CD25	TCR- α/β	TCR- γ	TCR- δ
Freshly isolated	1*	2	1	1	1	1	92	3	1	4	1	2	0
Incubated with:													
None	3	4	3	2	3	2	93	1	1	5	2	2	1
rIL-1 (50 U/ml)	2	4	4	1	1	2	92	1	1	8	3	ND	ND
rIL-2 (100 U/ml)	2	4	3	2	0	0	90	0	1	10	2	3	2
rsCD23 (25 ng/ml)	1	3	2	1	2	1	86	2	1	4	1	ND	ND
rIL-1 + rIL-2	2	5	3	2	2	2	89	1	2	9	2	ND	ND
rsCD23 + rIL-2	3	4	4	3	2	1	93	3	1	11	3	ND	ND
rsCD23 + rIL-1	6	53	45	23	37	7	95	2	0	34	39	4	3
rsCD23 + rIL-1 + rIL-2	7	59	51	21	33	8	97	1	1	33	39	5	4
rsCD23 + rIL-1 + anti-CD23	4	7	5	3	4	3	83	2	2	7	2	ND	ND

CD7+ cells isolated from human thymus. They were incubated for 48 h in medium containing 20% AB sera and various factors, as indicated. They were labeled thereafter with various mAbs.

* Mean from four experiments (SD < 15% of values obtained).

Finally, the effect of CD7⁺ cell preincubation with rsCD23, rIL-1, and rIL-2 on the induction of clonogenic cells in limiting dilution and the effect of anti-CD23 mAb addition were tested. As shown in Fig. 3, rsCD23 + rIL-1-supplemented cultures displayed a cloning frequency of 1/2.5, while other culture conditions remained inefficient in this respect. Addition of anti-CD23 to primary cultures drastically decreased the number of clonogenic cells.

Together, these results indicate that sCD23, synergistically with IL-1, is able to induce one of the first steps in T cell differentiation, which is the acquisition of CD2, CD25, CD3, CD4, CD8, and TCR- α/β expression by immature CD7⁺ precursors, although TCR repertoire of cell progeny was not defined.

CD23 was initially considered as a B cell differentiation marker (10). Subsequently, this molecule was shown to be identical to the low affinity receptor for IgE (Fc ϵ R2), which may also be expressed on monocytes/macrophages, eosinophiles, platelets, some T cells, and epidermal Langerhans cells (6). CD23 is a 45-kD membrane gly-

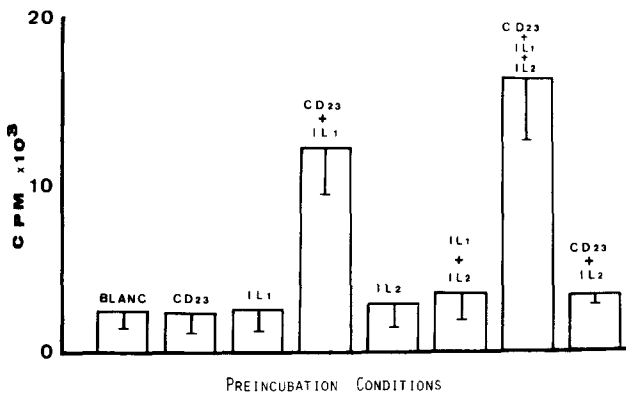


FIGURE 1. Proliferative response after in vitro conditioning of thymic CD7⁺ precursors. CD7⁺ cells were first incubated with various factors for 24 h (as in Table I). They were then washed and cultured (5×10^4 /well) with anti-CD21+III mAb (1/400 ascites) and 100 U/ml rIL-2. [³H]Thymidine (1 μ Ci) was added on day 5, and radioactivity uptake was measured on day 6. Results are mean \pm SD from four experiments, each done in triplicates.

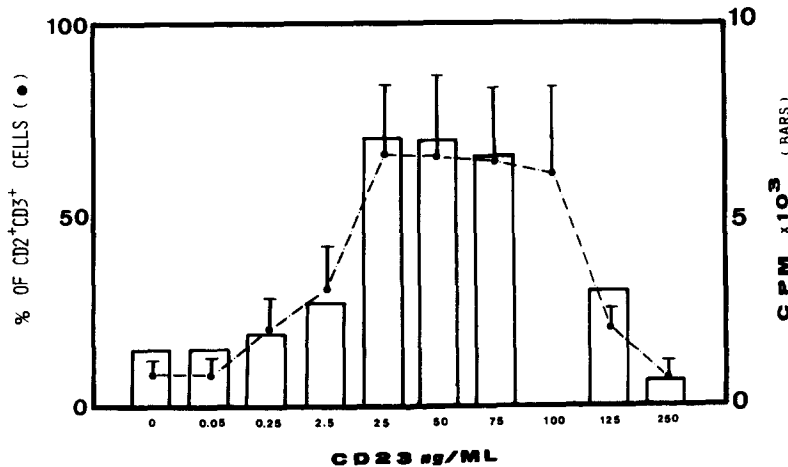


FIGURE 2. Responses of CD7⁺ precursors to increasing concentrations of rsCD23. CD7⁺ thymocytes were cultured with constant rIL-1 concentration (50 U/ml) and various rsCD23 doses (as in Table I), washed, and labeled with OKT11 (CD2) and OKT3 (CD3). The results show the percentage of precursors acquiring both CD2 and CD3 expression (●) (mean \pm SD from two experiments). The effects on the proliferative responses of the treated cells (as in Fig. 1) are also shown (bars) (mean from two experiments in triplicates; SD <25%).

coprotein that is cleaved into soluble fragments, and its expression is induced by IL-4 and suppressed by IFN- α (6). Soluble CD23 was reported to control the synthesis of human IgE (9), to regulate the proliferation of B (11) and T cells (12), and to promote early B cell differentiation (13). We previously reported the ability of supernatants from lectin-stimulated, enriched B cells to promote *in vitro* prothymocyte differentiation (3, 5). The present work indicates that sCD23, together with IL-1, have similar activity as they promote *in vitro* maturation of CD7⁺ prothymocytes into CD2-responsive cells. These factors seem to directly trigger prothymocytes. Indeed, epithelial and other thymic accessory cell depletion after treatment by CD14, CD58, and anti-MHC-II mAb and complement did not significantly reduce cell maturation.

Whether CD23 is expressed intrathymically remains to be established. A low number of CD23⁺ B lymphocytes have been observed in human thymus (14). As CD23 is expressed by other cells, it remains possible that thymic accessory cells, including epithelial cells, express CD23. In regard to IL-1, Le et al. (15) have documented IL-1 production by human thymic epithelium after *in vitro* incubation with autologous thymocytes. IL-1 is known to indirectly induce IL-2 responsiveness in mature thymocytes (19).

Although the respective roles of IL-1 and sCD23 remain to be determined, taken collectively, these data show that the first maturation steps during T cell development need factor(s) distinct from IL-2.

Summary

The ability of human thymus-derived CD7⁺CD2⁻CD3⁻ cells to acquire mature T cell antigens was assessed. Purified CD7⁺ thymocytes were incubated with rIL-1, rIL-2, and/or recombinant soluble CD23 (rsCD23). Short-term incubation of these cells with only rsCD23 + rIL-1 induced mature T cell antigen expression on at least half of the cells. The induction of CD2 was functionally significant, as these cells became able to respond to CD2 triggering and could proliferate in response to IL-2. Possible sources of CD23 in the thymus are under investigation.

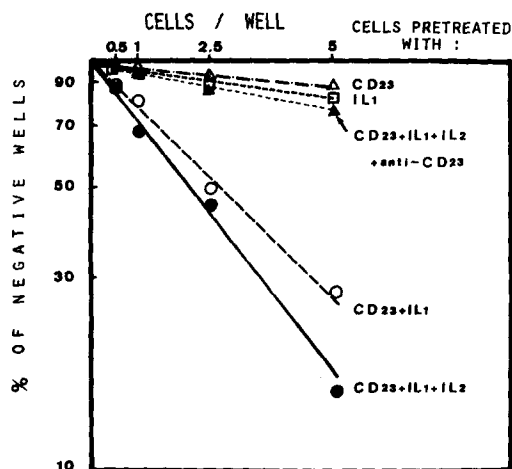


FIGURE 3. Synergistic effect of rsCD23 and rIL-1 on the cloning capacity of CD7⁺ precursors. The latter were incubated with rsCD23 (25 ng/ml), rIL-1 (50 U/ml), anti-CD23 (25 μ g/ml), and/or rIL-2 (100 U/ml) for 48 h, washed, and cultured in limiting dilutions in the presence of CD2_{I+III} and rIL-2. Positive vs. negative wells were scored from day 10 after cultures.

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