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Macrophage-T Cell Interaction Is Essential for the Induction of p75 Interleukin 2 (IL-2) Receptor and IL-2 Responsiveness in Human CD4$^+$ T Cells

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Fresh human CD8$^+$ T cells showed a strong proliferative response to a high concentration of interleukin 2 (IL-2) in the absence of macrophages. In contrast, CD4$^+$ T cells revealed no significant IL-2 responsiveness in the absence of macrophages. However, if CD4$^+$ T cells were cocultured with macrophages, they showed higher proliferative response to IL-2 than CD8$^+$ T cells. In accordance with the magnitude of IL-2 responsiveness, freshly isolated CD8$^+$ T cells expressed significant amounts of p75 IL-2 receptor, while fresh CD4$^+$ T cells did not express p75 IL-2 receptor. The expression of p75 IL-2 receptor on CD4$^+$ T cells was induced by coculture with macrophages. The macrophage-induced p75 IL-2 receptor acquisition was blocked by monoclonal antibody (mAb) against class II antigen. Moreover, the addition of anti-CD4 mAb or anti-class II mAb to the culture caused a great inhibition of IL-2 responsiveness of CD4$^+$ T cells. These results strongly suggest that macrophage-T cell interaction through CD4 and/or class II molecules is essential for the expression of p75 IL-2 receptor and IL-2 responsiveness in human CD4$^+$, but not CD8$^+$ T cells.

Key words: IL-2 — IL-2 receptor — IL-2 responsiveness — Human CD4$^+$ T cells — Macrophage

T lymphocytes are triggered by antigens through T3-TCR complex or by an alternative pathway and proliferate in response to interleukin 2 (IL-2[6]).[1-3] The production of monoclonal antibody (mAb) against p55 and p75 IL-2 receptors has made it possible to elucidate the mechanisms of IL-2-induced cell proliferation of immunoregulatory cells.[4-6] Initially, IL-2 was found to act only against activated T cells.[7] However, recent studies have demonstrated that high concentrations of IL-2 could elicit the proliferation of resting T cells and NK cells through p75 IL-2 receptor.[8,9] In particular, NK cells express large amounts of p75 IL-2 receptor and reveal higher IL-2 responsiveness than resting T cells do.[9] Although it has been shown that purified T cell populations (mixture of CD8$^+$ T cells and CD4$^+$ T cells) could respond to IL-2 in the absence of any stimulation and accessory cells,[10] it remains unclear whether purified CD4$^+$ T cells and CD8$^+$ T cells individually show similar responsiveness to IL-2 without any stimulation.

In order to clarify this issue, human CD4$^+$ and CD8$^+$ T cells were separated from peripheral blood mononuclear cells (PBMC) by FACStar and their responsiveness to recombinant IL-2[11] was determined. In accordance with previous results,[12] low concentrations of IL-2 (less than 100 U/ml) could not induce significant proliferative responses of purified T cells (data not shown). However, a high concentration of IL-2 (2,000 U/ml) could elicit a strong proliferative responses of isolated CD8$^+$ T cells. As shown in Fig. 1, CD8$^+$ T cells showed a marked proliferative response to IL-2, irrespective of the presence of macrophages. In contrast, purified CD4$^+$ T cells showed no significant proliferative response to IL-2 in the absence of macrophages. However, culture of CD4$^+$ T cells with macrophages resulted in a striking proliferation in response to IL-2. Interestingly, CD4$^+$ T cells revealed a higher proliferative response than CD8$^+$ T cells if they were cultured with macrophages. However, if CD4$^+$ T cells were separated from macrophages by using a Millicell (Japan Millipore Ltd.), which can pass soluble factors, but not cells, CD4$^+$ T cells did not respond to IL-2 (data not shown). IL-1 has been shown to have a capacity to regulate the IL-2 responsiveness.[13,14] However, the unresponsiveness of CD4$^+$ T cells to IL-2 in the absence of macrophages was not restored by the addition of IL-1. From these results, it was considered that direct contact between CD4$^+$ T cells and macrophages was essential for the induction of IL-2 responsiveness in human CD4$^+$ T cells. Aribia *et al.*[12] reported that purified CD4$^+$ and CD8$^+$ T cells could not respond to IL-2 unless monocytes were present. The

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[6] Abbreviations: IL-2, interleukin 2; mAb, monoclonal antibody; PBMC, peripheral blood mononuclear cells.

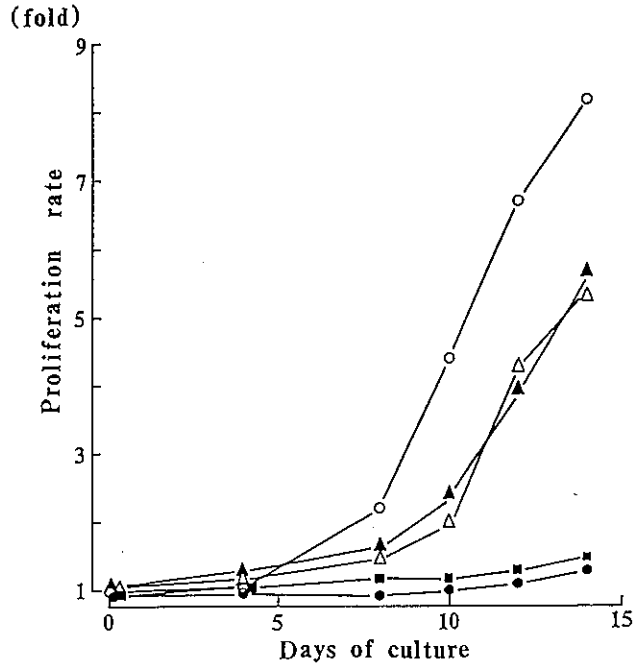

Fig. 1. Differential requirement of macrophages for IL-2 responsiveness of freshly isolated human $CD8^+$ and $CD4^+$ T cells. $CD4^+$ and $CD8^+$ T cells were separated from human PBMC by sorting with a FACStar (Becton Dickinson). Then, the isolated $CD4^+$ T cells or $CD8^+$ T cells (2×10^5) were cocultured with or without mitomycin C (30 μg/ml)-treated macrophages (2%) in the presence of 2,000 U/ml of recombinant human IL-2 (kindly donated by Takeda Pharmaceutical Co., Ltd., Osaka).[11] The plastic-adherent PBMC were used as a source of macrophages. IL-1 was kindly donated by Dainippon Pharmaceutical Co., Ltd., Osaka.[13] RPMI 1640 containing 10% heat-inactivated human AB serum, glutamine, penicillin, streptomycin, HEPES buffer was used for the tissue culture. After various periods of culture, the number of the cells was counted to determine the cell growth. The proliferation rate shown in the figure was calculated by means of the following formula: Proliferation rate=number of cells after culture/number of cells before culture. (●), $CD4^+$ T cell+IL-2; (○), $CD4^+$ T cells+macrophage+IL-2; (▲), $CD8^+$ T cells+IL-2; (△), $CD8^+$ T cells+macrophage+IL-2; (■), $CD4^+$ T cells+IL-2+IL-1 (200 U/ml).

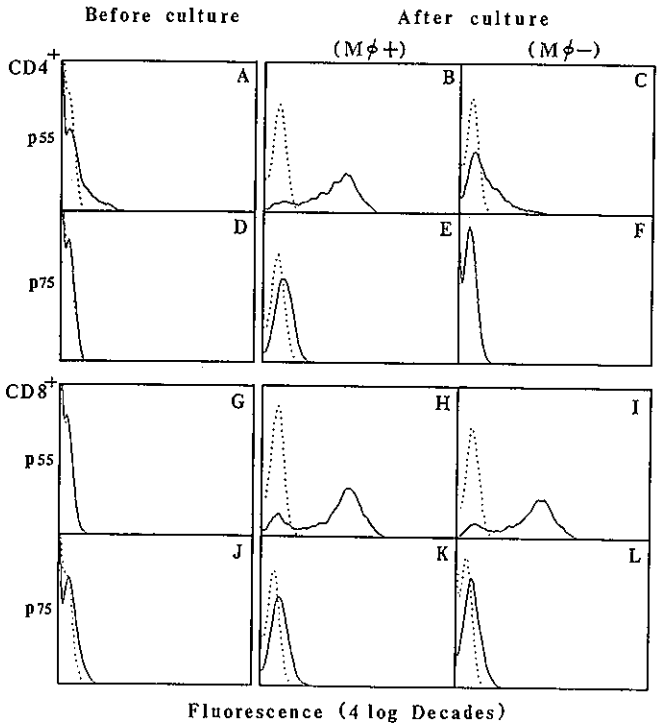

Fig. 2. The expression and induction of p55 and p75 IL-2 receptors on $CD4^+$ and $CD8^+$ T cells. $CD4^+$ and $CD8^+$ T cells were stained with PE-conjugated Leu-3a or Leu 2a and sorted by FACStar. The isolated cells were then stained with FITC-conjugated H-31 mAb[15] or TU27 mAb[5] to analyze p55 or p75 expression before culture (A, D, G, J). The sorted $CD4^+$ or $CD8^+$ T cells were cultured with (B, E, H, K) or without (C, F, I, L) macrophages (2%) in the presence of IL-2 for 7 days and their expressions of p55 (B, C, H, I) and p75 (E, F, K, L) were determined by FACScan (after culture). Dotted lines represent control curves and solid lines show the stained profiles.

discrepancy between our results and theirs appeared to be due to the different concentrations of IL-2 used for the experiments. We used 2,000 U/ml of IL-2, while they used 20 U/ml of IL-2. Our results were supported by the report of Bich-Thuy *et al.*,[10] who demonstrated that a high concentration of IL-2 was necessary for the induction of IL-2 responsiveness of purified T cells (mixture of $CD4^+$ and $CD8^+$ T cells) in the absence of accessory cells.

Recent studies have demonstrated that T cells express at least two kinds of IL-2 receptors, p75 and p55,[5, 15] and p75 IL-2 receptor is responsible for competent signal transduction, whereas p55 IL-2 receptor primarily functions to assemble a high-affinity receptor by association with p75.[8-10] Therefore, it was postulated that the discrepancy between IL-2 responsiveness of $CD4^+$ and $CD8^+$ T cells might be derived from different roles of p75 or p55 IL-2 receptor on $CD4^+$ and $CD8^+$ T cells. To assess this issue, we examined the expression of p55 and p75 IL-2 receptors on $CD4^+$ and $CD8^+$ T cells. In accordance with a previous report by our colleagues,[16] $CD8^+$ T cells expressed low but significant amounts of p75 IL-2 receptor, while the expression of p55 IL-2

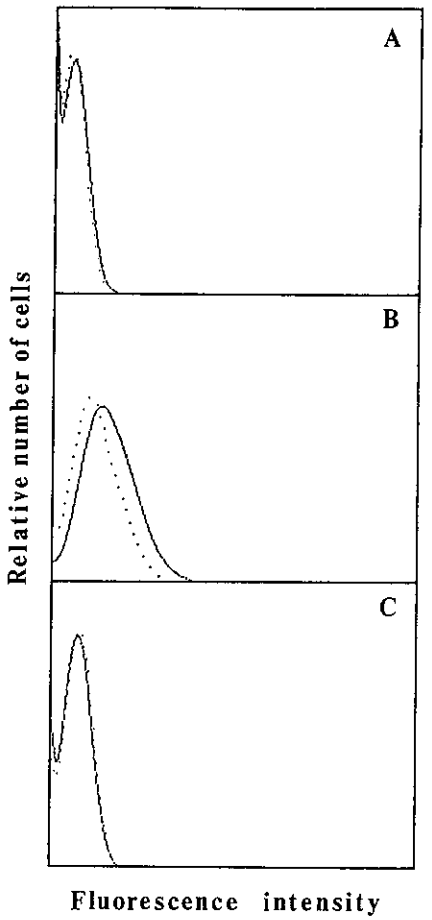

Fig. 3. Blocking of macrophage-induced p75 IL-2 receptor expression of CD4$^+$ T cells by mAb against class II antigen. CD4$^+$ T cells were cocultured with or without mitomycin C-treated macrophages in the presence or absence of mAb against class II antigen for 5 days. Then, the expression of p75 IL-2 receptor on the cells was determined by FACScan. (A), CD4$^+$ T cells cultured alone; (B), CD4$^+$ T cells cultured with macrophages; (C), CD4$^+$ T cells cultured with macrophages in the presence of mAb against class II antigen. Dotted lines represent control curves and solid lines show the stained profiles.

receptor was negligible. In contrast to this, CD4$^+$ T cells expressed p55 IL-2 receptor but the expression of p75 IL-2 receptor was not detectable (Fig. 2). The fact that CD8$^+$ T cells expressing only p75 IL-2 receptor could respond to IL-2 strongly suggested that p75, but not p55, IL-2 receptor was important in the initiation of IL-2 responsiveness of purified T cells, as previously proposed.[8-10] The unresponsiveness of CD4$^+$ T cells to IL-2 alone may be due to the lack of p75 IL-2 receptor expression in the unprimed condition. The expression of p75 IL-2 receptor became detectable on CD4$^+$ T cells cultured with macrophages plus IL-2. However, IL-2 alone could not induce the expression of p75 IL-2 receptor on CD4$^+$ T cells, though p55 expression was augmented. As shown in Fig. 3, p75 IL-2 receptor was also induced by coculture of CD4$^+$ T cells with macrophages in the absence of IL-2. Moreover, it was demonstrated that macrophage-induced p75 IL-2 receptor acquisition of CD4$^+$ T cells was completely blocked by the addition of mAb against class II (DR) antigen. These results indicate that macrophages are essential for the induction of p75 IL-2 receptor acquisition in human unprimed CD4$^+$ T cells.

As described above, soluble factors derived from macrophages could not replace macrophage-function to induce IL-2 receptor and IL-2 responsiveness. Therefore, the cell-cell interaction between macrophages and T cells was considered to be essential for the induction of IL-2 responsiveness of CD4$^+$ T cells. Besides T cell receptor complex, accessory molecules such as CD4, CD8, LFA-1 or CD2 have been shown to play an important role in immunoregulation.[17] To determine what molecules are important for macrophage-T cell interaction to induce IL-2 responsiveness of CD4$^+$ T cells, we tested the effect of various kinds of mAbs on IL-2-induced T cell proliferation in the presence of macrophages. As can be seen in Fig. 4, the IL-2 responsiveness of CD4$^+$ T cells was inhibited by the addition of mAb against CD4 or class II molecules (DR and DQ), while mAb against CD8 had no effect on the cell growth. In contrast to CD4$^+$ T cells, the IL-2 responsiveness of CD8$^+$ T cells was not affected by mAb against CD4, CD8, or class II molecules. The fact that mAb against IL-2 strikingly inhibited the cell growth of both CD4$^+$ and CD8$^+$ T cells ruled out the possibility that the cell growth of purified T cells was induced by some lymphokine other than IL-2 produced during coculture of T cells with macrophages. Because mAbs against CD4 and class II could not inhibit the IL-2 responsiveness of activated CD4$^+$ T cells (data not shown), it seemed to be unlikely that mAbs blocked IL-2 responsiveness of CD4$^+$ T cells by delivering negative signals through CD4 and/or class II molecules. Anti-T3 mAb can deliver the same signals as macrophages to induce IL-2 responsiveness of CD4$^+$ T cells (data not shown). Taking these results together, it appears that CD4$^+$ T cells may be activated by interacting with class II molecules on macrophages through T cell receptor complex and/or CD4.

It has been demonstrated that human T cells could respond to IL-2 in the absence of any stimulation and accessory cells.[10] However, the data described herein initially demonstrate that unprimed human CD4$^+$ and CD8$^+$ T cell subsets have differential triggering mechanisms for the initiation of IL-2 responsiveness. Macro-

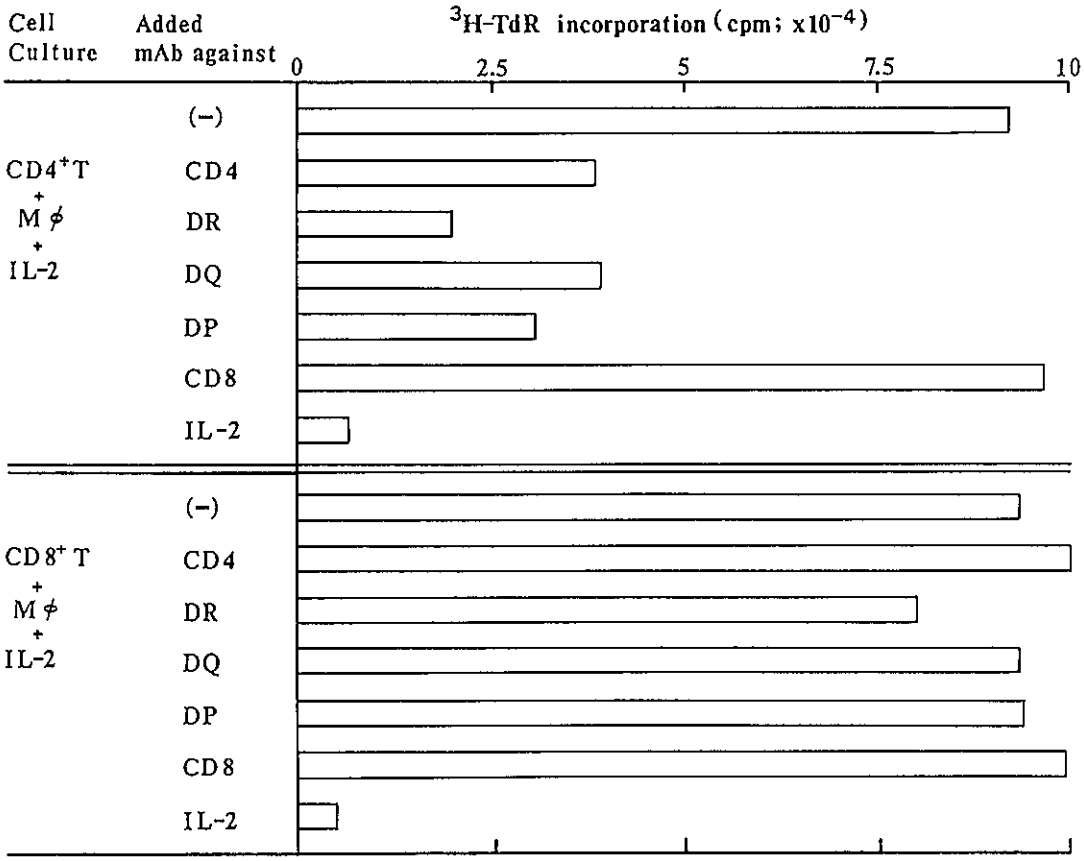

Fig. 4. Macrophage-T cell interaction via CD4 and class II antigen is essential for the initiation of IL-2 responsiveness of freshly isolated human CD4$^+$ T cells, but not CD8$^+$ T cells. FACStar-sorted CD4$^+$ or CD8$^+$ T cells (2×10^5 cells) were cocultured with macrophages (2%) in the presence of 2,000 U/ml of IL-2. mAb against CD4, CD8 or class II antigen (DR or DQ; kindly donated by Y. Nishimura, Kyushu University, Fukuoka) or IL-2 (Genzyme) was added to this coculture to determine the blocking effect on cell growth. Ten days after the start of culture, the cells were pulsed with ^{3}H-TdR (0.5 μCi/well) for 4 h and their proliferative response was measured.

phage-T cell interaction through CD4 and class II molecules seemed to be essential for the induction of p75 IL-2 receptor and IL-2 responsiveness in human CD4$^+$ T cells but not CD8$^+$ T cells.

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