Regulation of the Human Interleukin-2/Interleukin-2 Receptor System: A Role for Immunosuppression (43737)

RAYMOND KAEMPFER[1]

Department of Molecular Virology, The Hebrew University—Hadassah Medical School, Jerusalem 91120, Israel

Abstract. **The strength of the cellular immune response is regulated to a large extent by the amount of interleukin-2 (IL-2) produced in response to a stimulus. The ability of lymphocytes and other cells to respond to IL-2 depends upon the expression of cell surface IL-2 receptors. Formation of a high-affinity IL-2 receptor is regulated primarily through induction of its α subunit, IL-2Rα. Once formed, the IL-2Rα chain turns over rapidly, rendering expression of high-affinity IL-2 receptors during the immune response dependent upon continuous activity of the IL-2Rα gene. The induced expression of both human IL-2 and IL-2Rα chains is sensitive to cell-mediated suppression by CD8 cells; depletion of CD8 cells leads to extensive superinduction. This coupled suppression of IL-2 and IL-2Rα genes greatly increases the extent of control, and strongly limits the strength, of the signal transduced by this ligand/receptor system during an immune response.** [P.S.E.B.M. 1994, Vol 206]

Interleukin-2

Interleukin-2 (IL-2) has a central role in the cellular immune response, for it regulates the clonal expansion of activated T cells (1). The strength of the immune response is regulated to a large extent by the amount of IL-2 produced in response to a stimulus (1). IL-2 serves not only as the essential growth factor for all subsets of T lymphocytes but also acts on other cells in the immune system, including macrophages, B lymphocytes, natural killer cells, lymphokine-activated killer cells, and immature thymocytes (1–3). In the nervous system, oligodendrocytes respond to the growth-promoting activity of IL-2 (3). The ability of a cell to respond to IL-2 by proliferation requires the transient expression of the IL-2 receptor which is also induced by the immune stimulus (4, 5).

The IL-2 Receptor: Structure and Function

During an immune response, the ability of lymphocytes and other cells to respond to IL-2 is completely dependent upon the induced expression of high-affinity IL-2 receptors on the cell surface (for reviews, see Refs. 2, 3, 6–8). The high-affinity IL-2 receptor ($K_d = 10^{-11}$ *M*) is a heterotrimer, containing α-, β-, and γ-chains (Fig. 1). Possibly, additional polypeptides participate in this complex (6).

The α-chain (IL-2Rα, p55 or Tac Antigen) (9–11) by itself binds IL-2 with low affinity ($K_d = 10^{-8}$ *M*), while the β-chain (IL-2Rβ or p70/p75), and p64 γ-chain (12) together form an intermediate affinity receptor ($K_d = 10^{-9}$ *M*) (2, 3). The βγ heterodimer is responsible for internalization of IL-2 and for signal transduction (2, 3).

Normal, resting peripheral blood mononuclear cells (PBMC) express little, if any, IL-2Rα mRNA (9). Expression of the high-affinity IL-2 receptor thus depends strictly upon the induction of IL-2Rα gene expression by antigen or mitogen. IL-2Rβ expression is constitutive in CD8 cells but inducible in CD4 cells (2), increasing approximately 5- to 10-fold after mitogenic activation (13). Resting PBMC constitutively expresses IL-2Rγ mRNA (12).

The mature IL-2Rα chain is composed of 251

[1] To whom requests for reprints should be addressed at Department of Molecular Virology, The Hebrew University—Hadassah Medical School, Jerusalem 91120, Israel.

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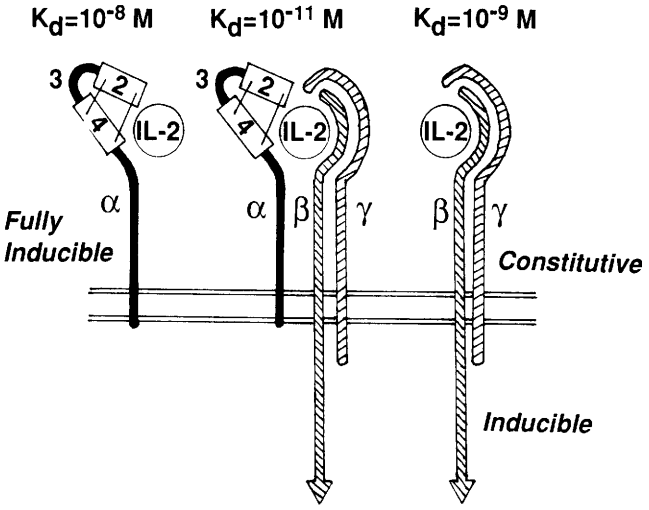

Figure 1. Schematic of the human IL-2 receptor. The functional receptor is composed of at least three polypeptide chains, α, β, and γ. Numbered regions on the IL-2Rα chain represent protein domains encoded by exons 2, 3, and 4, respectively.

amino acids encoded by eight exons, the signal peptide being encoded by exon 1 and the extracellular domain by exons 2–6 (9–11, 14). Exons 2–4 contribute the essential IL-2 binding domains in the IL-2Rα molecule (15). Segments encoded by exons 2 and 4 exhibit sequence homology; disulfide bridges stabilize the conformation of protein domains encoded by these exons, including two that connect between these regions (16) (Fig. 1). Both IL-2 and anti-Tac, a monoclonal antibody that blocks binding of IL-2, primarily recognize sites within the region encoded by exon 2, but both low- and high-affinity binding of IL-2 requires the domain encoded by exon 4 (15).

Rapid Turnover of the IL-2Rα Chain

Upon mitogenic induction, IL-2Rα chains appear on the cell surface, reaching maximal levels within 24 hr. The IL-2Rα chain continues to be expressed over a prolonged time interval; high levels of IL-2Rα chain persist for over 7 days, while soluble IL-2Rα chains become detectable only after 48 hr (4, 5, 17). This long-term expression of IL-2Rα chains on induced cells might suggest that after their synthesis, IL-2Rα protein molecules are quite stable and remain associated with the cell for an extended period of time before their release in soluble form. However, this is not the case. The IL-2Rα chain disappears rapidly from induced cells when translation is inhibited with cycloheximide (17). The half-life of IL-2Rα subunit protein is 2–3 hr, at least 50-fold shorter than the time interval over which IL-2Rα chains are expressed upon induction. The decline in cell-associated IL-2Rα is matched by a rapid decline in cell surface IL-2Rα and is not accompanied by any increase in soluble IL-2Rα protein. Long-term expression of the IL-2 receptor on the cell surface thus results from the continual synthesis and rapid breakdown of new IL-2Rα chains in the cell. Steady-state expression of IL-2 receptors after an immune stimulus hence depends upon continuous expression of the IL-2Rα gene. Indeed, the prolonged expression of IL-2Rα chains is accompanied by an equally sustained expression of IL-2Rα mRNA (17).

The rapid turnover of the unstable IL-2Rα chain provides a mechanism for finely attuned control of high-affinity IL-2 receptor expression. Instability of the IL-2Rα chain allows for a prompt shut-off of responsiveness to IL-2 in the course of a cellular immune response, upon cessation of IL-2Rα gene transcription. Instability of receptor chains may serve more generally to effect rapid changes in ligand-receptor interactions.

Suppression of IL-2 and IFN-γ Gene Expression

Induction of human IL-2 and IFN-γ genes by mitogens such as phytohemagglutinin (PHA) requires *de novo* transcription and results in the appearance of a wave of mRNA (18–20). Both expression of mRNA (20, 21) and biological activity of IL-2 and IFN-γ (20–23) can be superinduced by exposing cells, before their induction, to low doses of γ-irradiation, a treatment thought to prevent activation of cells with suppressive properties (22, 24, 25). The amplitude of the induced waves of IL-2 and IFN-γ mRNA is increased upon γ-irradiation, yet the shape of this wave remains unchanged (20, 21). These findings suggested that γ-irradiation-sensitive cells have a role in regulating expression of IL-2 and IFN-γ genes. Indeed, concomitant with the induction of IL-2 and IFN-γ genes, mitogenic stimulation induces a transient activation of cells that themselves do not express these genes, but possess the ability to effectively inhibit IL-2 and IFN-γ gene expression in other cells from the same population (21).

Human CD8 cell lines were isolated with antigen-nonspecific suppressive properties that inhibit CD4 cell proliferation (26). Depletion of CD8 cells, thought to include cytotoxic and mature suppressor T cell populations (27, 28), led to greater activation of T helper cell function (29). Early studies involved measurement of biological responses that are the cumulative result of a sequence of events (26–29), leaving open the mechanism of this control.

If expression of the IL-2 gene is sensitive to inhibition by cells with suppressive properties, then removal of such cells should enhance IL-2 gene expression. Indeed, depletion of CD8 cells leads to a 10-fold superinduction of IL-2 activity (Fig. 2A). CD8 cells constitute 10%–20% of the original cell population, rendering it unlikely that this extensive increase in IL-2 production was due merely to removal of IL-2-utilizing cells. Indeed, depletion of CD8 cells leads to

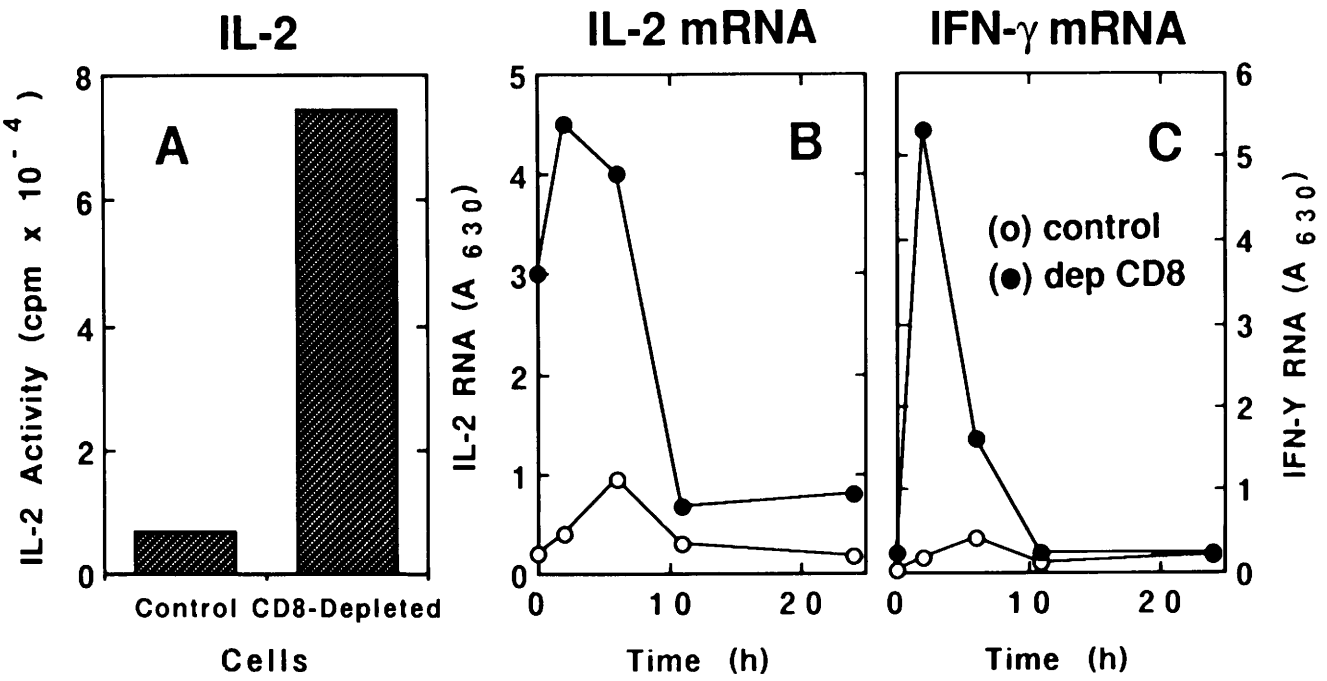

Figure 2. Superinduction of IL-2 and IFN-γ gene expression in CD8-depleted lymphocyte populations. (A) Control and CD8-depleted tonsil cell populations were induced with PHA for 24 hr and IL-2 activity was assayed in culture medium. Depletion was by complement lysis. (B, C) Nonadherent PBMC (○), or CD8-depleted cells derived from them (●), were induced with PHA; at the times indicated, RNA was quantitated by dot blot hybridization with IL-2 (B) and IFN-γ (C) [^{32}P]labeled antisense RNA transcripts. Depletion was with CD8-coated immunomagnetic beads; CD8 cells comprised 11% of the total cell population. (Reproduced from Ref. 21 with permission.)

a commensurate increase in the amplitude of the induced waves of IL-2 and IFN-γ mRNA (Fig. 2B and 2C) (21). These increases are far too rapid and extensive to be explained by an effect on T cell proliferation. CD8 cells thus are capable of suppressing the induced expression of IL-2 and IFN-γ genes which occurs primarily in Th1 type CD4 cells.

In a total lymphoid cell population, the extent of expression of IL-2 and IFN-γ genes will depend upon the balance between the activities of cells that express or suppress these genes. Transient expression of human IL-2 and IFN-γ genes results from dynamic interaction between these cell subpopulations. Mitogenic stimulation induces an activation of cells with suppressive activity that shuts off the expression of IL-2 and IFN-γ genes concomitantly induced in other cells, suppression becoming dominant over expression in the course of induction. Indeed, induction of IL-2 and IFN-γ mRNA largely precedes the appearance of suppressive activity in the total cell population, allowing expression of these genes to occur before a dominant down-regulation is exerted (21).

The temporary nature of suppressive cell activity does not diminish its critical role in regulating the strength of a cellular immune response, but indeed, allows for a rapid return to the untriggered state. This cell-mediated suppression thus constitutes a normal, indeed essential feature of the cellular immune response.

Suppression of IL-2Rα Gene Expression

The regulation of the IL-2 gene by cell-mediated suppression raised the question whether IL-2Rα gene

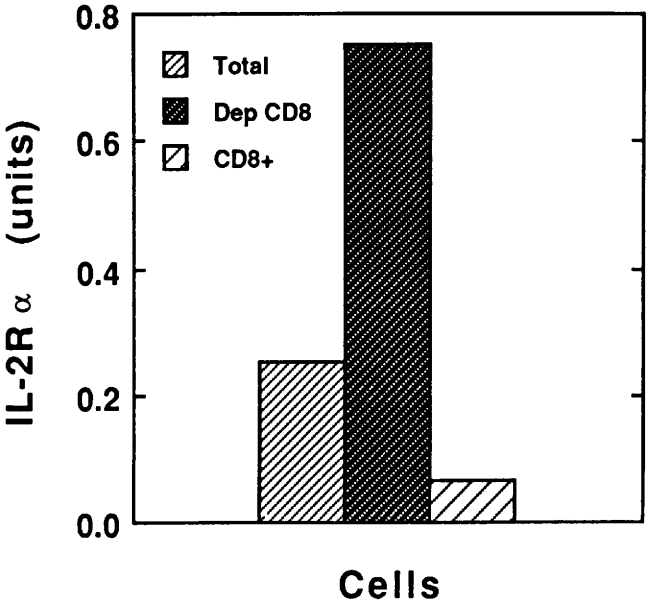

Figure 3. Superinduction of IL-2Rα chain expression in CD8-depleted lymphocyte populations. Control tonsil cells, CD8 cells derived from them, and the CD8-depleted population were induced with PHA for 21 hr. Cell-associated IL-2Rα chains were quantitated (49). Depletion was with CD8-coated immunomagnetic beads; CD8 cells comprised 18% of the total cell population (Sayar D, Ketzinel M, Arad G, Gerez L, Deutsch E, Nussinovich R, Kaempfer R, submitted).

expression might also be subject to such suppression. Indeed, depletion of CD8 cells leads to superinduction of cell-associated IL-2Rα chains in the remaining cell population (Fig. 3). The 3-fold increase in IL-2Rα chains in the CD8-depleted cells actually represents a 5-fold superinduction when corrected for IL-2Rα expression by the CD8 cell subset in the total cell population and for the reduced number of cells in the CD8-depleted population. This result shows that CD8 cells actively suppress expression of the IL-2Rα chain during induction (Sayar D, Ketzinel M, Arad G, Gerez L, Deutsch E, Nussinovich R, Kaempfer R, submitted).

Relative to CD8-depleted cells, isolated CD8 cells express low amounts of IL-2Rα (Fig. 3). It is interesting that CD8 cells actively suppress expression of the IL-2Rα chain in the non-CD8 cell population, yet they themselves can express this chain.

Clearly, suppression of IL-2Rα chain expression will result in a reduction in high-affinity IL-2 receptors. It is not yet known if expression of the IL-2Rβ chain, inducible in CD4 cells (2), is also regulated by suppression. However, since formation of high-affinity IL-2 receptors depends totally on the induction of the short-lived IL-2Rα chain, it is especially sensitive to any inhibition of this process.

Coupled Suppression of IL-2 and IL-2Rα Genes: Implications for Regulation of the Cellular Immune Response

The coupled suppression of IL-2 and IL-2Rα genes greatly increases the extent of control that acts to limit the strength of the signal transduced by the IL-2/IL-2 receptor system during a cellular immune response. First, by preventing IL-2 gene expression in induced Th1 cells, concomitantly activated CD8 cells effectively suppress the amount of IL-2 elicited in response to a stimulus (Fig. 2). Second, through a similarly strong suppression of IL-2Rα chain expression (Fig. 3), CD8 cells severely restrict the number of high-affinity IL-2 receptors displayed on the surface of the responding T cell. The resulting reduction in the number of functional IL-2 receptors will decrease the ability of that cell to respond to IL-2, particularly since the IL-2Rα chain is highly unstable and thus must be synthesized continuously at a high rate in order to maintain responsiveness to IL-2 (17). This rapid turnover renders IL-2Rα gene expression even more sensitive to suppression.

The powerful CD8 cell-mediated suppression ensures that IL-2 and IL-2Rα genes are normally expressed to no more than a small proportion of their full potential, making their expression particularly sensitive to regulation by external signals. Any condition, including a pathological state, that alters the balance between cells that actively express the IL-2 and/or IL-2Rα genes on one hand, and cells that actively suppress these genes on the other, will profoundly affect the extent of T cell proliferation and amplification during an immune response. Similarly, the effect of any agent that selectively prevents or promotes the activation of either expressing or suppressing cell subpopulations will be greatly amplified by the coupled suppression of this ligand/receptor pair of genes.

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