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Regulatory Counteraction to IL-2 Activity

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A rational approach to suppression of cellular immune responses requires detailed information on the mechanisms that regulate induction and differentiation of the effector cells involved. Recent research has led to a far more advanced knowledge of the T-cell activation process and in particular to the recognition of the central role of soluble mediators, in particular Interleukin-2 (IL-2). It appears that T-cell activation comprises a cascade of interdigitating mechanisms, some of which are antigen-specific and others of which are not. A theory has been elaborated by several authors that combines both antigen-specific and nonspecific events.^(1,2) This theory implies that an antigen-specific initial stimulus drives T-cells into an activated state, whereas further differentiation and clonal expansion of T-cells is regulated by antigen-nonspecific mediators, such as IL-2. The production of the latter is concomitantly initiated in the course of a cellular immune response. In Figure 1 we tried to depict the present concept for T-cell activation that is inspired by the results of several groups.⁽¹⁻⁶⁾

It can be seen from this figure that the mediator IL-2, previously has been named T-cell Growth Factor (TCGF),⁽⁷⁾ plays a central role in the generation of effector function by expanding activated cells.

In view of the regulatory events accompanying an immune response this model requires antagonistic forces that either inactivate and/or functionally restrain IL-2 activity. Consequently several attempts have been made to investigate the mechanisms which accomplish this function.

Most of the present information has been obtained by applying strategies that are known to lead to suppression of immune responses, such as lectin-activated suppressor cells,⁽⁸⁻¹⁰⁾ lectin- or antigen-induced suppressive factors,^(11,12) serum-derived suppressive activities,⁽¹³⁾ or immunosuppressive drugs.⁽¹⁴⁻¹⁷⁾

The purpose of this article is to summarize briefly the results that have been obtained in this particular field of research and finally to put forward an extended (still tentative) model for T-cell activation (see Figure 2). No attempt is made to be exhaustive, especially with regard to including all references that had been relevant for the generation of the theory on T-cell activation outlined in Figure 1. Therefore review articles are cited and those may be consulted for the work that contributed to this theory.

SUPPRESSOR CELLS

In recent publications by Northoff et al.,⁽⁹⁾ Gullberg et al.,⁽⁸⁾ and Palacios and Möller,⁽¹⁰⁾ it has been proposed that the production and function of IL-2 is regulated by suppressor cells.

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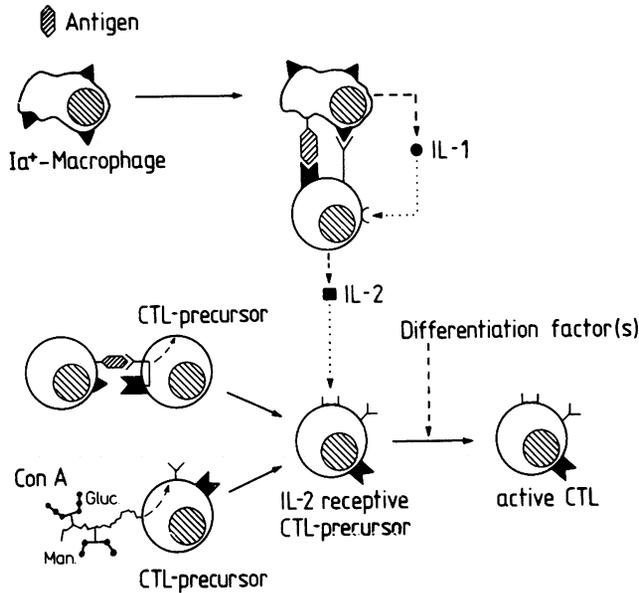


FIG. 1. The role of IL-1 and IL-2 in T-cell activation. The activated macrophage releases IL-1 and presents antigen to the T_H cell. The IL-2 released by the T_H cell acts upon the IL-2 receptive CTL-precursor. The CTL-precursor acquires IL-2 responsiveness by antigen specific or mitogenic activation. Differentiation factors are required in parallel to IL-2 for maturation to effector function.

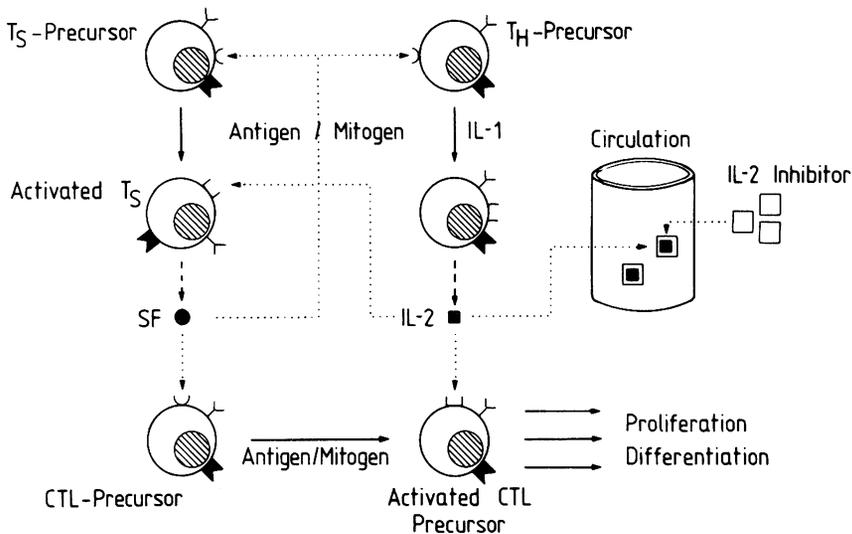


FIG. 2. Schematic description of functional antagonists to IL-2 during T-cell activation. SF is produced by an activated suppressor cell and arrests the precursor cells of T_H cells, T_S cells, and CTLs before they acquire sensitivity to IL-2. IL-2 that is not consumed is neutralized by the IL-2 inhibitor in the circulation.

Northoff et al.⁽⁹⁾ described that fresh mononuclear leucocytes from human peripheral blood secreted very few or no IL-2 in the culture medium when stimulated with mitogen or antigen. Preincubation of these cells for 2-5 days *in vitro* rendered them capable of producing and secreting increasing amounts of IL-2. Addition of increasing numbers of fresh leucocytes to such cultures gradually reduced IL-2 release. The suppressive effect was associated with the T-cell fraction and the authors concluded that shortlived suppressor T-cells prevent the production of IL-2 in fresh leucocyte cultures.

Gullberg et al.⁽⁸⁾ observed that IL-2 production in ConA stimulated murine spleen cell cultures shows a phasic time course with maximum production after 18 hr and a subsequent sharp decline, and postulated an active mechanism that limits IL-2 production. The decrease of IL-2 release (i) was not due to consumption of essential medium components, (ii) to inactivation of accessory cells, nor (iii) to activation of the lectin, (iv) was not caused by inhibitory substances that interfere directly with IL-2, and (v) cannot be explained by a feedback mechanism depending on previously produced IL-2. ConA activated spleen cells when transferred to a second culture exerted a dose dependent reduction of the *de novo* IL-2 production. Thus, they concluded that ConA induced suppressive cells account for the reduced IL-2 release from cells after 18-24 hr *in vitro* culture.

Since ConA activated T-cells are the adequate responder- and consumer-celi population for IL-2^(19,20) the observed reduction of the IL-2 titer may in part be explained by absorption of IL-2 to its specific membrane receptors.^(3,21)

This was shown by Palacios and Möller⁽¹⁰⁾ who found that ConA activated human T-cells cause a marked suppression of PHA induced proliferation or of alloantigen induced cytolytic activity of leucocytes. The suppression was abrogated by addition of IL-2 to the detection system. Furthermore, when ConA activated suppressor cells were exposed to IL-2 prior to the addition to the detection system (i) they absorbed IL-2, and (ii) were functionally inactivated by IL-2. The authors therefore concluded that at least part of the suppressive activity of such cells is due to the reduction of the available IL-2.

SUPPRESSIVE FACTOR(S)

T-cell activation *in vitro* by lectins or antigen is followed by the generation of suppressor cells⁽²³⁾ as well as by the release of suppressive mediators^(23,24) into the culture medium. We isolated suppressive factor(s) from the supernatants of alloantigen stimulated murine spleen cells and tested their interference with T-cell activation.^(11,12) A factor(s) was separable from IL-2 by gel-filtration that inhibited the response of T-cells to alloantigen, as well as the generation of regulatory T-cells from their precursors when added at the beginning of the *in vitro* culture. Furthermore this factor(s) prevented the release of IL-2 from producer T-cells but had no detectable effect on the interaction of IL-2 with receptive T-cells. These experiments suggest that suppressive factor(s) do not directly interfere with IL-2 function but modulate early events in T-cell activation (see Figure 2) very likely by suppression of IL-2 release and by preventing T-cells to acquire IL-2 responsiveness. The main characteristics of this suppressive mediator(s) are summarized in Table 1.

We presently focus on the further biochemical purification and characterisation of the molecular species, which depends on large scale production of active supernatants under serum free conditions. Further studies with the at least semipurified factor are required to define the lineage and differentiation stage of its target cell, the mechanisms underlying the suppressive activity and its possible role *in vivo*.

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TABLE 1. PROPERTIES OF SF

Molecular weight	10 000 d (as estimated from gel filtration)
Elution from DEAE-Sephrose	180 mM NaCl in 0.01 M Tris/HCl pH 8.0
Temperature stability	85% activity lost after 60 min at 70°C
pH stability	79% activity lost after 6 h at pH 4.0 29% activity lost after 6 h at pH 10.0
Induction	Antigen-specific, by T-cell mitogens
Lymphoid target cell	T-cell
Inhibition of ³ H-Thymidine uptake by T-cells	Yes
Inhibition of CTL-induction (only when added early)	Yes
Inhibition of IL-2 release	Yes
Interference with IL-2 activity on T-cell blasts	No
Producer cell	Not yet identified

Data from references 11, 12, and 18.

Suppressor factor(s) could be released independently from IL-2. If however the concentration of IL-2 regulated the release of SF this should be considered in feasible therapeutical applications of IL-2.

SERUM-DERIVED INHIBITOR(S) OF IL-2 ACTIVITY

Hardt et al.⁽¹³⁾ attempted to identify antagonists to the nonrestricted and nonspecific activity of IL-2 and found that normal mouse serum contains high levels of an IL-2 inhibitor with an apparent molecular weight of 55 000 daltons. A molecule(s) of this size interfered directly with IL-2 and its mitogenic function on receptive T-cells. The inhibitory function was neither antigen-specific nor H-2 restricted. The actual titer of the inhibitory molecule seemed to be under the control of T-cells, since after cell transfer of allogeneic cyclophosphamide sensitive Lyt 2,3⁺ T-cells that induce a graft versus host reaction in nude mice, high serum levels of IL-2 inhibitor activity were found.

INFLUENCE OF PHARMACOLOGICAL AGENTS ON IL-2 RELEASE AND FUNCTION

Several authors have reported on the effects of immunosuppressive drugs on IL-2 release and function. Gillis et al.^(16,17) analyzed whether glucocorticoids exert their suppressive effect on T-cell activation either at the level of the activated and IL-2 sensitive T-lymphocyte or at the level of IL-2 release. Mitogen induced IL-2 production and T-cell proliferation were completely inhibited by pharmacologic concentrations of dexamethasone, whereas the proliferation of splenocytes in the presence of maximum inhibitory doses of dexamethasone was restored by addition of IL-2. They concluded that a major pathway of glucocorticoid mediated immunosuppression leads to functional inhibition of the IL-2 producing cell.

Larsson⁽¹⁵⁾ further studied the T-cell activation process using dexamethasone and in addition the microbial product Cyclosporin A, a cyclic octadecapeptide isolated from *Cyclindrocarpum lucidum* (CyA), which has selective immunosuppressive effects on T-lymphocytes.^(14,25,26) CyA inhibited the acquisition of the lectin-induced responsiveness to IL-2 in resting T-cells at concentrations that did not affect the production of IL-2. CyA did not inhibit the IL-2 dependent division of T-cell blasts in response to IL-2, thus in-

dicating that it did not interfere with the binding of IL-2 to its surface receptors nor with the subsequent response to the mitogenic signal.

In contrast to CyA, dexamethasone at pharmacologic concentrations (10^{-5} to 10^{-7} M) (i) significantly reduced IL-2 production, (ii) but did not prevent T-lymphocytes to acquire responsiveness to IL-2, and (iii) left IL-2 mediated proliferation of T-cell blasts unaffected. Furthermore, Larsson states that the reduced response of normal spleen cells to ConA in the presence of dexamethasone could be overcome to some extent by the addition of IL-1 isolated from the WEHI-3 macrophage cell line. This indicates that dexamethasone at nontoxic concentrations primarily interferes with the macrophage dependent production of IL-1, which then results in reduced IL-2 release. This is in accordance with earlier studies⁽²⁷⁾ showing that the macrophage metabolism is very sensitive to treatment with glucocorticoids.

SUMMARY AND CONCLUSIONS

Summarizing the above mentioned findings it appears that T-cells after activation go through several stages of differentiation. During these stages they are susceptible to either activating or inhibitory factors, which restrict the response to regulatory mediators to a limited period.

Taken together the data suggest that the release and function of IL-2 is regulated by several mechanisms (Figure 2):

- (i) IL-2 leaving the primary site of T-cell activation and entering systemic circulation may be inactivated by an IL-2 inhibitor present in the serum of normal mice.
- (ii) IL-2 liberated during T-cell activation can be absorbed by T-cells (T-suppressor cells) that are activated in parallel and that express the appropriate surface receptors.
- (iii) The *de novo* production of IL-2 is suppressed by suppressor cells and/or soluble mediators thereof.
- (iv) Generation of further IL-2 responsive T-cells is prevented by inhibition of T-cells during the early phase of the activation process (either directly by suppressor T-cells and/or via suppressive factors(s)).

The principal mechanisms that could negatively regulate the effects of IL-2 are listed as follows:

- (i) Reduction of preformed IL-2 activity (ConA activated T-(suppressor-)cells, serum derived IL-2 inhibitor).
- (ii) Reduction of IL-2 production and release (ConA activated suppressor T-cells, alloantigen induced suppressive factors(s), CyA at high concentrations, dexamethasone via reduction of IL-1).
- (iii) Inhibition of the acquisition of the IL-2 responsive state (CyA at lower concentrations, alloantigen induced suppressive factor(s)).

Recent results reported by several groups⁽²⁸⁻³¹⁾ indicate that the T-cell activation process, and especially T-cell differentiation to active cytotoxic T-lymphocytes does in fact require further mediators in addition to IL-1 and IL-2. This was concluded from studies showing that IL-2 alone (derived from a T-cell hybridoma cell line) causes proliferation but is not sufficient to give rise to active CTL in accessory cell depleted T-cell cultures. However, a mixture of IL-2 and IL-2 depleted conditioned medium from spleen cells resulted in full differentiation to active CTL.⁽²⁸⁾ Further studies will have to clear the role of physiological and pharmacological immunosuppressants in the network of factors controlling T-cell proliferation and differentiation.

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