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MODULATION OF IL-2- AND IL-4-INDUCED CYTOTOXICITIES IN HUMAN T HELPER LYMPHOCYTE CLONES BY TUMOR NECROSIS FACTOR-α[1]

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A set of alloreactive IL-2-dependent human CD4$^+$ 45RA$^-$w29$^+$56$^-$ Th cell clones was divided into two groups according to their ability to respond to IL-4 by proliferation and their susceptibility to inhibition by TNF-α. The latter cytokine blocked proliferative responses to IL-2 of IL-4-nonresponsive clones, but did not affect proliferation of IL-4-responsive clones. In the present communication, it is demonstrated that exposure of apparently noncytotoxic Th cells to IL-4 resulted in the dose-dependent induction of allospecific CTX in clones previously shown to be capable of responding to IL-4 by proliferation. In contrast, IL-2 induced both allospecific and MHC-unrestricted "NK-like" CTX in both IL-4 responder and nonresponder TCC. However, coculture with IL-4 in addition to IL-2 downregulated this induction of NK-like CTX by the IL-2 (in those clones capable of responding to IL-4). Acquisition of these two types of CTX by the same TCC was additionally modulated by TNF-α, which also blocked the induction of NK-like CTX but had no effect on the induction of allospecific CTX by either IL-2 or IL-4. In contrast, IFN-γ was unable to block induction of either type of CTX in this model system. These data suggest that even at the clonal level, the relative availability of a number of different up- and down-regulatory cytokines influences the outcome of an immune response. In the present model, IL-2 up-regulates specific and NK-like CTX, the latter component of which is down-regulated by TNF-α or IL-4, whereas IL-4 itself can up-regulate specific but not NK-like CTX.

B cells demonstrate functional flexibility during the evolution of an immune response by generating Ag receptor variants and thus facilitating selection of antibody-effector molecules of higher affinity and better specificity (1). Unlike B cells, Ag receptor genes of T cells do not undergo somatic mutation during the course of their clonal expansion in an immune response (2). However, this does not necessarily mean that their central guiding role in the immune response remains inflexibly fixed after being laid down at some point in ontogeny, presumably during thymic selection. There are indications from experiments in vitro that functional modulations of several kinds occur even at the monoclonal level in models of T cell immune responses of the simplest kind. Perhaps the best defined of these involve a number of different but related reports on the induction of clonal anergy preventing further clonal expansion but not necessarily completely preventing all functions (e.g., lymphokine secretion) of the T cells (3–7). This type of effect is unique in that it has also been shown to occur in vivo (e.g., in models of adult tolerance induction) (8).

In addition to the above, there is a range of reports on alterations of clonal T cell functions in vitro, either as an apparently "spontaneous" result caused by undefined factors acting in long term cultures (9–12), or as a result of deliberate modification of culture conditions (13, 14). Several of these model systems have dealt with the acquisition of CTX[3] by originally apparently non-CTX "helper" T cells. For example, age-related acquisition of both MHC-unrestricted ("NK-like") CTX in man and mouse (9, 10) or of allospecific CTX in man (11) has been described, as well as IFN-α-stimulated induction of CTX in human TCC (14). That helper T cell clones possess potential lytic machinery even under conditions in which they do not spontaneously utilize it is emphasized by findings that "bridging" to target cells with appropriate antibody results in lysis of the targets (15). In a different system, lack of appropriate stimulation has also been described which results in acquisition of new functions by TCC, as demonstrated by the acquisition of syngeneic CTX activity by mouse allospecific CTX clones cultured with growth factors but without cellbound alloantigen (16). Even variables such as the density of cells in culture may radically alter their measured functions (17), and this in turn could explain some of the controversial findings in limiting dilution precursor frequency studies not demonstrating single hit kinetics (18). Many of these reports on functional flexibility of T cells could be explained by hypothesizing that T cell function does not remain fixed, but even at the clonal level is strongly influenced by the microenvironment. Cytokine modulation of the functions mediated by T cell clones then becomes a good possibility for explaining the control of this kind of functional flexibility.

In a continuing analysis of T cell functional flexibility, the present report concerns the induction and modulation by IL-2, IL-4, and TNF-α of CTX activity in a relatively homogeneous set of CD4$^+$ alloreactive human

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[3] Abbreviations used in this paper: CTX, cytotoxic; LAK, lymphokine activated killing; LCL, lymphoblastoid cell line; TCC, T cell clone; TCGF, T cell growth factor; LU, lytic units.

helper T cell clones. These were derived from three normal donors' PBMC stimulated in vitro with irradiated cells from the same HLA-Dw5-homozygote, and were all capable of allospecific autocrine proliferation and B cell help, but under usual conditions of propagation were not CTX. However, they could be divided into two groups based on their responsiveness, or lack of it, to stimulation by phorbol ester and calcium ionophore (19). Moreover, they also differed from one another in that only 5 of 15 could use IL-4 instead of IL-2 as an exogenous growth factor, and that, unlike IL-4 nonresponders, proliferation of such IL-4 responders could not be blocked by TNF-α (20). The present report concerns the effects of exposure to these cytokines on the induction of CTX in these non-CTX cells.

MATERIALS AND METHODS

TCC and cultures. Three IL-4-responder and three IL-4-nonresponder clones (258-15, 257-14, 250-7, and 257-6, 257-5, 256-7, respectively) were studied (20). These lines all manifested monoclonal TCR gene rearrangements by Southern blotting (6) (F. Kalthoff, unpublished observations). The terms IL-4 responders and nonresponders relate to the ability of these TCC, all of which were derived by limiting dilution from MLC by using purified IL-2 as exogenous growth factor, to proliferate when cultured with IL-4 instead of IL-2 (20). All clones were maintained mycoplasma-free (4-wk agar culture test, University of Tübingen Hygiene Institute, Tübingen, FRG) in RPMI 1640 supplemented with 10% human serum and 20 U/ml IL-2 (Lymphocult T-HP, Biotest Co., Frankfurt, FRG) and stimulated once per week with irradiated allospecific LCL. TCC were removed from maintenance cultures 4 days after stimulation and exposed to higher concentrations of the cytokines IL-2 (Biotest), rIL-4 (10^8 U/mg from Genzyme, IC Chemikalien, Munich, FRG), rTNF-α (2×10^7 U/mg, Genzyme) and rIFN-γ (2×10^7 U/mg, Bioferon, Laupheim, FRG). Cells were harvested for use after a week's culture in titrated amounts of these cytokines or combinations thereof.

TCGF bioassays. The IL-4-responder clone 258-15 was used as an indicator cell 4 days after antigenic stimulation in a bioassay for TCGF. Titrated amounts of test supernatant were added to 1×10^4 TCC/well in the presence or absence of a saturating concentration (ascites 1/100) of a CD25 mAb (TÜ69), which blocks essentially all IL-2 responses (20). IL-2 and IL-4 standard control curves were established in each experiment. The [^{3}H]TdR incorporation after 2 days in the presence of TÜ69 was taken as an estimate of content of TCGF other than IL-2 and the difference in proliferation with and without TÜ69 as an indication of IL-2 content. These two estimates thus assume that the T cells do not respond to any other factors such as IL-7, which, however, is not completely excluded. Assuming that IL-4 is responsible for all of the non-IL-2 TCGF activity, the sensitivity of this clone-based assay is <1 U/ml of IL-4.

CTX. Standard ^{51}Cr-release assays were used throughout, and results were expressed either as percent specific isotope release, or as lytic units, where 1 LU = number of effectors cells per 10^7 required for 25% specific isotope release from 2×10^3 target cells (21). Target cells were from the NK-susceptible K562 erythroleukemia cell line, the NK-resistant Daudi Burkitt lymphoma line, or from EBV-transformed normal HLA-D homozygous LCL.

RESULTS

Production of and response to TCGF by TCC. It was previously demonstrated (20) that the alloreactive IL-2-dependent TCC studied here could be divided into two groups on the basis of their proliferative responses to IL-4 given as an exogenous growth factor, and their inhibition by TNF-α (which correlated with IL-4-nonresponsiveness). By using a bioassay in which the response of an IL-2- and IL-4-reactive clone was blocked by an anti-IL-2R mAb, the non-IL-2 content of supernatants from stimulated TCC was estimated. Table I shows a comparison of TCC IL-2 and IL-4 production estimated in this way: 7/15 TCC were found to secrete both, and whereas none produced IL-4 alone, the remaining 8 produced IL-2 but not bioactive IL-4. Of the 7 TCC that secreted non-IL-2 TCGF, 2 failed to respond to IL-4, and none of the other 8 clones responded to it (Table I).

TABLE I

Production of and response to TCGF to CD4$^+$ Th-TCC[a]

TCC	Production		Response	
	IL-2	IL-4	IL-2	IL-4
233-7	23	21	46	23
248-3	31	26	31	6
250-7	42	33	62	39
257-6	19	24	21	5
257-12	20	40	48	36
257-14	46	45	53	17
258-15	37	50	70	57
8 others	31	<1	44	4

[a] For TCGF production, 5×10^5 TCC were stimulated with PHA and LCL. After 24 h, supernatants were collected and assayed on clone 258-15. Data are presented as units per milliliter derived from standard curves of IL-2 and IL-4, where the response of indicator cells in the presence of anti-IL-2R mAb TÜ69 is taken to reflect IL-4 content, and the difference between the response with and without TÜ69 is taken as an estimate of IL-2 content. For measuring proliferative responses to IL-2 and IL-4, TCC were cultured with 20 U/ml of IL-2 or 1000 U/ml of IL-4 3 days after antigenic stimulation. Data are shown as median cpm ($\times 10^{-5}$) of triplicate cultures.

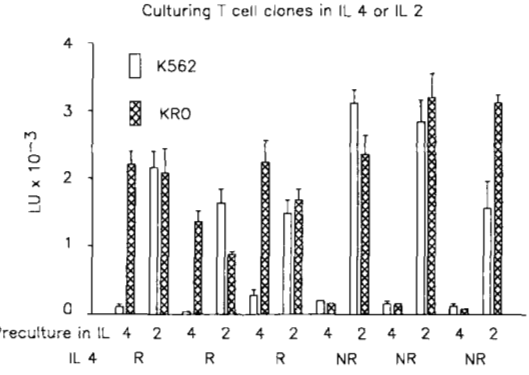

Figure 1. Acquisition of CTX by non-CTX TCC after culture with IL-2 or IL-4. CTX expressed as LU on K562 (NK-susceptible) or KRO-LCL (allospecific) target cells, mediated by IL-4 responder (IL 4 R) or IL 4 nonresponder (IL 4 NR) TCC after 6 days culture with 1000 U/ml IL-4 (preculture in IL 4) or 1000 U/ml IL-2 (preculture in IL 2). Data are LU + SEM. Clones were (from left to right) 258-15, 257-14, 250-7, 257-6, 257-5, 256-7.

Acquisition of CTX by TCC in high concentrations of IL-2 or IL-4. Three IL-4 responders and three nonresponders, as defined in Table I, were selected for further study. They were removed from maintenance culture (in 20 U/ml of IL-2) and transferred to cultures with 1000 U/ml of either IL-2 or IL-4, or put back into culture with the same concentration of 20 U/ml IL-2. After 7 days, they were harvested and titrated onto K562 targets to measure NK-like CTX or onto KRO-LCL targets to measure allospecific CTX. Figure 1 displays the results of one of two such experiments. On the left-hand side of the figure, three IL-4 responders are shown; on the right-hand side, three nonresponders. Preculture of these six clones in 1000 U/ml of IL-2 resulted in acquisition of CTX for both targets (Fig. 1), whereas the cells from the routine culture in 20 U/ml were not detectably CTX (data not shown). In contrast, culture of IL-4 responders with 1000 U/ml of IL-4 resulted in acquisition of lysis on KRO but not K562 targets, with no CTX induced in IL-4 nonresponders (Fig. 1). Titrations of IL-2 or IL-4 indicated that induction of CTX was maximal at the concentrations employed above, but was also detectable at rather lower concentrations (Fig. 2).

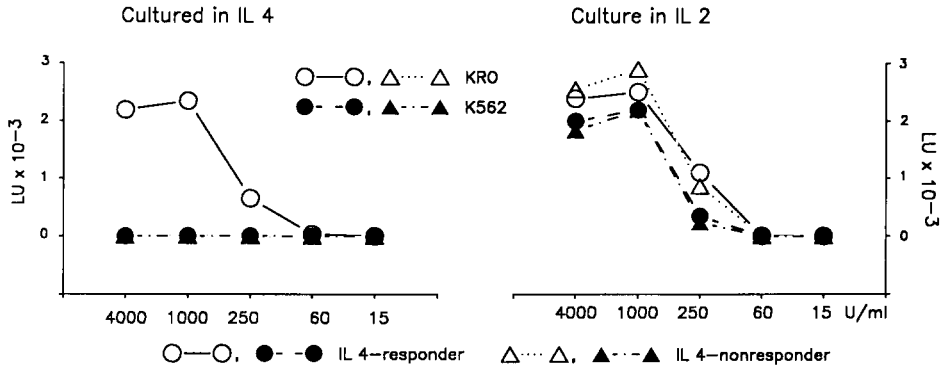

Figure 2. Induction of CTX in one IL-4-responder and one IL-4-nonresponder by titrated amounts of IL-2 or IL-4. TCC were assayed after 6 days incubation with IL-4 (left-hand panel) or IL-2 (right-hand panel).

Influence of cytokines on the IL-2-stimulated acquisition of MHC-unrestricted CTX. To assess possible interactions between cytokines in this model, TCC were precultured for 7 days with 1000 U/ml IL-2 to which was added either IL-4, IFN-γ or TNF-α (Fig. 3). These results confirm the induction of NK-like CTX for K562 by IL-2 and show that this is not inhibited (nor consistently enhanced) by 100 U/ml IFN-γ, although some enhancement was seen in one IL-4 nonresponder (not statistically significant). In contrast to the inaction of IFN-γ, TNF-α consistently and strongly blocked the acquisition of NK-like CTX by all six clones, regardless of their IL-4-responder status. This was not associated with a decreased recovery of viable cells after the 7-day culture period, whereas cell yields of the IL-4 nonresponders, where proliferation is inhibited by TNF-α (20), were considerably reduced (Table II). Numbers of the latter were adjusted to give the same E:T ratios as for the IL-4-responders in the CTX assays.

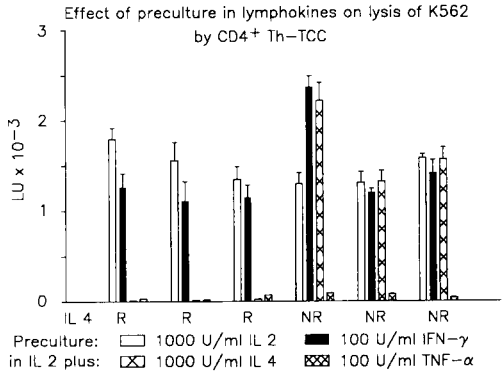

Figure 3. Effect of cytokines on induction of NK-like CTX by IL-2. IL-4 responder and nonresponder TCC (IL 4 R, NR) were cultured for 6 days with 1000 U/ml of IL-2 only, or IL-2 plus 1000 U/ml IL-4, 100 U/ml IFN-γ or 100 U/ml TNF-α, and then titrated onto K562 target cells.

TABLE II

Recoveries of viable cells after culture for 6 days with IL-2 ± TNF-α[a]

TCC	IL-4 responder	Recovery after Culture in IL-2 Plus	
		IL-4	TNF-α
250-7	Yes	160	93%
257-14	Yes	210	105
258-15	Yes	168	89
256-7	No	96	25
257-5	No	105	32
257-6	No	89	18

[a] TCC were cultured with 1000 U/ml of IL-2 or with IL-2 + IL-4 (1000 U/ml) or IL-2 + TNF-α (100 U/ml). Viable cells were enummerated after 6 days and results expressed as percent recovery of viable cells (IL-2 cultures = 100%).

Unlike TNF-α, IL-4 blocked the induction of NK-like CTX only by those clones that were capable of responding by proliferation to this lymphokine (Fig. 3). This was also not associated with decreased cell viability. On the contrary, cell yields for IL-4-responders were increased in the presence of IL-4 (Table II). One IL-4 nonresponder clone did show a somewhat enhanced response to IL-4 in this assay: this was the same clone that was also enhanced by IFN-γ (Fig. 3).

To ascertain whether these modulatory effects of the cytokines were peculiar to CTX against a target that was easy to lyse, the experiments were repeated on an NK-resistant target cell, Daudi. The results were almost exactly comparable to those obtained with K562 target cells, even concerning the unexpected induction of CTX by IL-4 and IFN-γ in the IL-4-nonresponder clone (data not shown).

Influence of cytokines on the IL-2-induced acquisition of allospecific CTX. Clones cultured with 1000 U/ml of IL-2 gained lytic activity for their allospecific target LCL, KRO, as well as for another HLA-DRw11 homozygous LCL, but not for HLA-DR1 or DR2 cells (data not shown). It was therefore examined whether induction of allospecific CTX, like induction of MHC-unrestricted CTX, could be down-regulated by cytokines. However, neither IFN-γ nor TNF-α was able to block acquisition of specific CTX (Fig. 4). Because IL-4 could by itself induce allospecific CTX (Fig. 1), its lack of inhibition of IL-2-induced allospecific CTX was to be expected (Fig. 4), but, again, the effect of TNF-α on inhibition of proliferation of IL-4 nonresponder clones was not reflected in inhibition of CTX induction.

Effects of higher and lower TNF-α concentrations. In order to clarify whether the induction of allospecific CTX

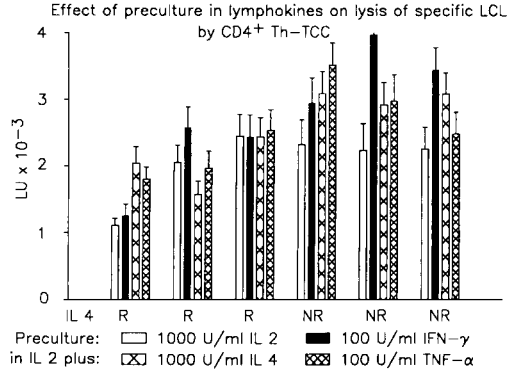

Figure 4. Effect of cytokines on induction of allospecific CTX by IL-2. Experiment as in Fig. 3 legend, but target cells were KRO-LCL.

was more resistant to inhibition than the induction of MHC-unrestricted CTX, cells from cultures containing 1000 U/ml of TNF-α were tested. Data in Figure 5 from an experiment with two TCC (one IL-4R and one IL-4NR) indicate that even this high concentration of TNF-α was unable to block the induction of allospecific CTX by IL-2. In addition, Figure 5 shows that concentrations of TNF-α of <100 U/ml still failed to enhance IL-2-stimulated induction of MHC-unrestricted CTX; rather, the inhibitory effect of the TNF-α titrated out gradually.

DISCUSSION

The present results demonstrate a dose-dependent induction by IL-4 of specific CTX in human $CD4^+$ helper TCC which were otherwise not detectably CTX. Only those clones that responded to IL-4 as well as IL-2 as an exogenous growth factor were found to acquire CTX under the influence of IL-4. Two clones that secreted TCGF other than IL-2 but failed to respond to IL-4 by proliferation also failed to respond by CTX acquisition. Even the highest concentration of IL-4 employed did not induce MHC-unrestricted CTX. In contrast to the specific effects of IL-4, high concentrations of IL-2 induced both specific and MHC-unrestricted, LAK-like CTX in all the TCC. The specific CTX induced by IL-2 was not caused indirectly by IL-2-stimulated IL-4 release because it was observed in all of the clones, regardless of their ability to secrete and/or respond to IL-4.

The induction of the NK-like CTX was down-regulated by concurrent addition of IL-4 (in IL-4 responders), a finding with direct parallels to the generation of LAK cells from fresh PBMC in humans (22, 23). This may imply that during an immune response in which predominantly IL-2-secreting cells are stimulated, NK-like activity would be encouraged, whereas secretion of IL-4 would both directly stimulate specific CTX as well as down-regulate MHC-unrestricted CTX in that fraction of TCC with IL-4 responsiveness. The report that very few TCC isolated in IL-4- rather than IL-2-mediated NK-like CTX on K562 is consistent with these findings (24).

Further experiments on cytokine modulation of CTX induction in these helper TCC demonstrated that TNF-α suppressed IL-2-stimulated acquisition of NK-like CTX, regardless of the clones' IL-4 responder status. Titrating the TNF-α did not reveal a lower (or higher) concentration

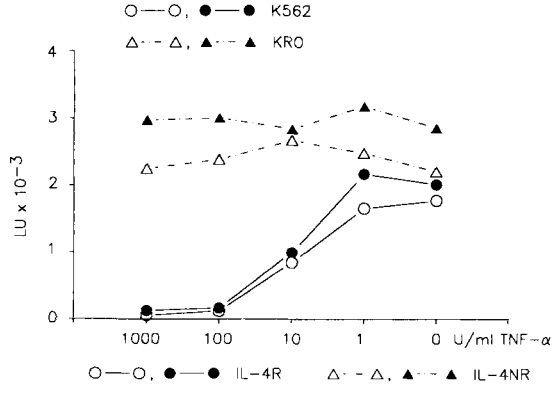

Figure 5. Titration of TNF-α. Cells from clone 257-14 (IL-4R) or 257-5 (IL-4NR) were cultured with 1000 U/ml of IL-2 and titrated amounts of TNF-α. After 6 days, cell numbers were adjusted and titrated onto ^{51}Cr-labeled NK targets (K562) or allospecific targets (KRO). Data are shown as LU. SEM are omitted for clarity of presentation.

at which enhancement of CTX was observable. This contrasts with the reported enhancing effect of TNF-α on LAK induction in PBMC (25). However, PBMC represent a mixture of different cells, whereas the TCC tested here were a homogeneous set of $CD4^+$ helper cells. However, this finding is also different from the effect of TNF-α on IL-2-driven proliferation of the same clones, where only IL-4 nonresponders were susceptible to inhibition (20). In contrast, TNF-α did not block the induction of allospecific CTX at all, again regardless of the IL-4 responsiveness of the TCC. These differences presumably reflect differences in signals required for the induction of CTX as opposed to proliferation.

These results may imply that the presence of TNF-α could further encourage the development of specific CTX during an ongoing immune response by 1) preventing clonal expansion of IL-4 nonresponders that can acquire only NK-like CTX, and 2) blocking IL-2-induction of NK-like CTX even in TCC responsive to IL-4. Because all the clones secrete TNF-α themselves after stimulation (26), evolution within longer term uncloned T cell cultures in the direction of specific CTX would be predicted from the present results. This seems not to have been directly tested, although the acquisition of specific CTX by non-CTX alloproliferative human TCC in long-term culture has been previously noted (11). Furthermore, at least in mouse long-term clones, mutually exclusive T cell subsets producing either IL-2 or IL-4 can be defined, whereas the patterns of cytokine secretion in mouse short term or in human clones tend not to be so sharply distinguishable (27). Regarding the TCC investigated here, all of the IL-4 responder clones also secreted TCGF activity attributed to IL-4, because their supernatants enhanced proliferation of IL-4-responder clone 258-15 in the presence of anti-IL-2R antibody TÜ69. There may also be a third group of TCC that secretes non-IL-2 TCGF without responding to IL-4, but these might be producing factors such as IL-7, which appears to be a "general" TCGF (28). All of these clones also secreted IL-2, so they do not fit into the murine Th1-vs-Th2 categories.

Several previously noted alterations in specificity and function of T cell clones may also be explicable in terms of the above findings. For example, the rapid but variable "degeneration" of specificity toward NK-like activity in some mouse TCC (29) could reflect the relative availability of IL-4, IL-2, and TNF-α in the cultures, where exposure even for only 1 wk to high concentrations of IL-2 but low IL-4 and TNF-α could be sufficient to alter TCC behavior. Differences between clones in their IL-2 or IL-4 secretory capacity might also help to explain the acquisition of specific CTX by some clones (11) but NK-like CTX by others (9).

The reactivities of Th cells to cytokines in the microenvironment during an immune response might thus have a significant immunoregulatory role. The induction of allospecific CTX measured here would be expected to apply also to Ag-specific CTX, where lysis of APC by Th cells (previously reported in mouse (30)) would inhibit further amplification of the immune response. This phenomenon, coupled with the finding that IL-4 enhances the generation of (non-CTX) specific suppressive activity of bulk lymphocyte populations in MLC (31), might further suggest that a function of IL-4 (at least in the T cell

compartment) is to *limit* the extent of an immune response.

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REFERENCES

1. **Kishimoto, T., and T. Hirano.** 1989. B lymphocyte activation, proliferation and immunoglobulin secretion. In *Fundamental Immunology.* 2nd ed. W. E. Paul, ed. Raven Press, New York, p. 385.
2. **Davis, M. M., and P. J. Bjorkman.** 1988. T-cell antigen receptor genes and T-cell recognition. *Nature 334:395.*
3. **Levich, J. D., D. E. Parks, and W. O. Weigle.** 1985. Tolerance induction in antigen-specific helper T cell clones and lines in vitro. *J. Immunol. 135:873.*
4. **Ceredig, R., and G. Corradin.** 1986. High antigen concentration inhibits T cell proliferation but not interleukin 2 production: examination of limiting dilution microcultures and T cell clones. *Eur. J. Immunol. 16:30.*
5. **Lamb, J. R., B. J. Skidmore, N. Green, J. M. Chiller, and M. Feldmann.** 1983. Induction of tolerance in influenza virus-immune T lymphocyte clones with synthetic peptides of influenza hemagglutinin. *J. Exp. Med. 157:1434.*
6. **Pawelec, G., T. Brocker, F. W. Busch, H.-J. Bühring, N. Fernandez, E. M. Schneider, and P. Wernet.** 1988. "Tolerisation" of human T-helper cell clones by chronic exposure to alloantigen: culture conditions dictate autocrine proliferative status but not acquisition of cytotoxic potential and suppressor-induction capacity. *J. Mol. Cell. Immunol. 4:21.*
7. **Mueller, D. L., M. K. Jenkins, and R. H. Schwartz.** 1989. Clonal expansion versus functional clonal inactivation: a costimulatory signalling pathway determines the outcome of T cell antigen receptor occupancy. *Annu. Rev. Immunol. 7:445.*
8. **Rammensee, H.-G., R. Kroschewski, and B. Frangoulis.** 1989. Clonal anergy induced in mature $V\beta6^+$ T lymphocytes on immunizing *Mls*-1^b mice with Mls-1^a expressing cells. *Nature 339:541.*
9. **Pawelec, G., E. M. Schneider, and P. Wernet.** 1986. Acquisition of suppressive activity and natural killer-like cytotoxicity by human alloproliferative "helper" T cell clones. *J. Immunol. 136:402.*
10. **Simon, M. M., H. U. Weltzien, H-J. Bühring, and K. Eichmann.** 1984. Aged murine killer T-cell clones acquire specific cytotoxicity for P815 mastocytoma cells. *Nature 308:367.*
11. **Fleischer, B.** 1984. Acquisition of specific cytotoxic activity by human T4+ T lymphocytes in culture. *Nature 308:365.*
12. **Chen, L-K., A. Bensussan, G. F. Burns, B. Tourvieille, A. Soulié, and M. Sasportes.** 1986. Allospecific proliferative human T-cell clones acquire the cytotoxic effector function after three months in culture, in IL-2 conditioned medium. *Hum. Immunol. 17:30.*
13. **Brooks, C. G., M. Holscher, and D. Urdal.** 1985. Natural killer activity in cloned cytotoxic T lymphocytes: regulation by interleukin 2, interferon, and specific antigen. *J. Immunol. 135:1145.*
14. **Chen, L.-K., D. Mathieu-Mahul, F. H. Bach, J. Dausset, A. Bensussan, and M. Sasportes.** 1986. Recombinant interferon-alpha can induce rearrangement of T cell antigen receptor alpha-chain genes and maturation to cytotoxicity in T lymphocyte clones in vitro. *Proc. Natl. Acad. Sci. USA 83:4887.*
15. **Spits, H., H. Yssel, J. Leeuwenberg, and J. E. DeVries.** 1985. Antigen-specific cytotoxic T cell and antigen-specific proliferating T cell clones can be induced to cytolytic activity by monoclonal antibodies against T3. *Eur. J. Immunol. 15:88.*
16. **Claesson, M. H., and R. G. Miller.** 1985. Functional heterogeneity in allospecific cytotoxic lymphocyte clones. II. Development of syngeneic cytotoxicity in the absence of specific antigenic stimulation. *J. Immunol. 134:684.*
17. **Melchers, I., and R. Rzepka.** 1988. Plasticity of T cell function. I. Cloned EL-4 lymphoma cells may help or suppress a primary antibody response depending on their own concentration and the assay system. *J. Immunol. 141:2873.*
18. **Eichmann, K., K. Fey, R. Kuppers, I. Melchers, M. M. Simon, and H. U. Weltzien.** 1983. Network regulation among T cells; conclusions from limiting dilution experiments. *Springer Semin. Immunopathol. 6:7.*
19. **Pawelec, G., F. Busch, U. Reinhold, A. Rehbein, I. Balko, and H-J. Bühring.** 1988. Division of human helper T cells into two sets on the basis of the induction of anti-tumor cytotoxicity by phorbol ester and calcium ionophore. *Eur. J. Immunol. 180:1147.*
20. **Pawelec, G., A. Rehbein, K. Schaudt, and F. W. Busch.** 1989. IL-4-responsive human helper T cell clones are resistant to growth inhibition by tumor necrosis factor-α. *J. Immunol. 143:902.*
21. **Pawelec, G., M. R. Hadam, A. Ziegler, J. Lohmeyer, A. Rehbein, I. Kumbier, and P. Wernet.** 1982. Long-term culture, cloning and surface markers of mixed leukocyte culture-derived human T lymphocytes with natural killer-like cytotoxicity. *J. Immunol. 128:1892.*
22. **Spits, H., H. Yssel, X. Paliard, R. Kastelein, C. Figdor, and J. E. De Vries.** 1988. IL-4 inhibits IL-2-mediated induction of human lymphokine-activated killer cells, but not the generation of antigen-specific cytotoxic T lymphocytes in mixed leucocyte cultures. *J. Immunol. 141:29.*
23. **Pawelec, G., E. Schneider, G. Ehninger, A. Rehbein, and H. Schmidt.** 1989. Partial correction of defective generation of lymphokine-activated killer cells in patients with chronic myelogenous leukaemia after in vivo treatment with interferon-α (Wellferon). *Cancer Immunol. Immunother. 29:63.*
24. **Paliard, X., H. Yssel, D. Blanchard, J. A. Waitz, J. E. DeVries, and H. Spits.** 1989. Antigen specific and MHC nonrestricted cytotoxicity of T cell receptor $\alpha\beta^+$ and gammaδ^+ human T cell clones isolated in IL-4. *J. Immunol. 143:452.*
25. **Chouaib, S., J. Bertoglio, J.-Y. Blay, C. Marchiol-Fournigault, and D. Fradelizi.** 1988. Generation of lymphokine-activated killer cells: synergy between tumor necrosis factor and interleukin 2. *Proc. Natl. Acad. Sci. USA 85:6875.*
26. **Pawelec, G., K. Schaudt, A. Rehbein, and F. W. Busch.** 1989. Differential secretion of tumor necrosis factor-α and granulocyte/macrophage colony stimulating factors but not interferon-γ from $CD4^+$ compared to $CD8^+$ human T cell clones. *Eur. J. Immunol. 19:197.*
27. **Mosmann, T. R., and R. L. Coffman.** 1989. Heterogeneity of cytokine secretion patterns and functions of helper T cells. *Adv. Immunol. 46:111.*
28. **Grabstein, K. H., A. E. Namen, K. Shanebeck, R. F. Voice, S. G. Reed, and M. B. Widmer.** 1990. Regulation of T cell proliferation by IL-7. *J. Immunol. 144:3015.*
29. **Wilson, A., and K. Shortman.** 1984. Degradation of specificity in cytolytic T cell clones: mouse strain dependence and interstrain transfer of nonspecific cytolysis. *Eur. J. Immunol. 14:951.*
30. **Ju, S.-T., R. H. DeKruyff, and M. E. Dorf.** 1986. Inducer T-cell-mediated killing of antigen-presenting cells. *Cell. Immunol. 101:613.*
31. **Pawelec, G., P. Baumgartner, A. Rehbein, U. Reusch, and K. Schaudt.** 1990. Cytokine regulation of the balance between alloindifferent and allospecific suppressor induction in mixed lymphocyte cultures. *Transplantation 49:615.*