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Review

Retinoic acids regulate apoptosis of T lymphocytes through an interplay between RAR and RXR receptors

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Abstract

Vitamin A deficiency has been known for a long time to be accompanied with immune deficiency and susceptibility to a wide range of infectious diseases. Increasing evidence suggests that retinoic acids derived from vitamin A are involved in the functional regulation of the immune system. Of the two groups of retinoid receptors, retinoic acid receptors (RARs) and retinoid X receptors (RXRs) all-*trans* and 9-*cis* retinoic acids are high affinity ligands for RARs and 9-*cis* retinoic acid additionally binds to RXRs. In cells, at high concentrations, all-*trans* retinoic acid can be converted to 9-*cis* retinoic acid by unknown mechanisms. Apoptosis plays a major role in shaping the T cell repertoire and one way in which retinoids may affect immune functions is to influence the various apoptosis pathways. Indeed, it has been shown that retinoic acids can induce apoptosis, increase the rate of dexamethasone-induced death and inhibit activation-induced death of thymocytes and T lymphocytes. Therefore, retinoids together with glucocorticoids may be involved in regulating positive and negative selection of T lymphocytes. Here we demonstrate that retinoids can induce apoptosis of T cells through the stimulation of RARγ. Specific stimulation of RARα, on the other hand, prevents both RARγ-dependent and TCR-mediated cell death. In all these functions 9-*cis* retinoic acid proved to be more effective than all-*trans* retinoic acid suggesting the involvement of RXRs. Based on these results a possible mechanism through which costimulation of RARs and RXRs might affect spontaneous and activation-induced death of T lymphocytes is proposed.

Keywords: T lymphocytes; apoptosis; signal transduction; retinoic acid; tissue transglutaminase; fas ligand

Abbreviations: APC, antigen presenting cells; fasL, fas ligand; RA, retinoic acid; RAR, retinoic acid receptor; RXR, retinoid X receptor; TCR, T-cell receptor; TNF, tumour necrosis factor

Apoptosis induction via distinct signalling pathways shapes the T cell repertoire

Apoptosis or active cell death, plays an essential role in shaping the T lymphocyte repertoire which may influence the control of the body's immune responses. Lymphocytes differentiating in the thymus randomly generate their TCRs and become selected in the $CD4^+CD8^+$ stage. Those cells which express potentially autoreactive TCR undergo apoptosis after interacting with the antigen presenting cells (APC) (Jenkinson *et al*, 1989). This selection process can be studied using T cell hybridomas (Ucker *et al*, 1989) and can be mimicked by stimulating the TCR-associated CD3 molecule with a specific antibody (Smith *et al*, 1989) or by simultaneous addition of phorbol-dibutyrate and Ca^{2+}-ionophore *in vitro* which mimicks the TCR-induced signal transduction pathway (Iseki *et al*, 1991). Those cells which express functionally acceptable TCR become positively selected and differentiate into mature $CD4^+$ or $CD8^+$ single positive thymocytes (Groettrup and von Boehmer, 1993). In these cells bcl-2, an apoptosis inhibitory protein (Hockenbery *et al*, 1991) becomes upregulated and protects cells against death (Sentman *et al*, 1991; Szondy, 1997). However, the majority of T cells express functionally unacceptable TCRs and enter the apoptotic programme through a 'default pathway' which is accelerated when cells are exposed to high levels of glucocorticoids (Cohen, 1993), or by treatments leading to DNA breaks – ionizing radiation, 2-chlorodeoxyadenosine addition or inhibition of topoisomerase II by etoposide (Osborne *et al*, 1994; Ming-Sun *et al*, 1994; Szondy, 1995). The apoptotic programme induced in each of these cases works via different signal transduction pathways (Osborne, 1994). Stimulation of TCR and CD3 induces changes in second messenger systems (McConkey *et al*, 1992), glucocorticoids bind to steroid receptors, while inhibition of topoisomerase II or irradiation causes direct DNA damage. Each of these pathways appear to induce distinct sets of genes. The transcripts RP-2 and RP-8 are expressed in thymocytes following treatment with glucocorticoids (Owens *et al*, 1991). DNA damage leads to *p53* induction (Lowe *et al*, 1993; Szondy, 1995); thymocytes lacking *p53* are resistant to the lethal effects of ionizing radiation or etoposide, but not to other treatments (Lowe *et al*, 1993; Clarke *et al*, 1993). The immediate-early gene, *nur 77*, on the other hand, is induced in response to TCR signals but not by glucocorticoids or ionizing radiation (Liu *et al*, 1994). Antisense inhibition of the expression of *nur 77* prevents apoptosis in TCR stimulated cells but not if death was induced by other stimuli (Liu *et al*, 1994).

Mature T lymphocytes entering the periphery are relatively resistant to various apoptosis inducing signals due to the constant expression of bcl-2 (Yang and Korsmeyer, 1996; Szondy, 1997). After mitogenic stimulation, however, their

proliferation and survival depends on IL-2 production (Duke and Cohen, 1986) which upregulates bcl-X_L, another apoptosis inhibitory protein (Yang and Korsmeyer, 1996). After clearance of antigen, in the absence of further antigenic stimulation IL-2 production is declined and many of the lymphocyte clones die due to the lack of survival signal (mechanism of immune down-regulation). In the case of chronic activation, on the other hand, the limitation of the immune response occurs by a different mechanism. TCR stimulation leads to fasL and TNF production, and these cytokines initiate the apoptotic programme in the fas or TNF receptor bearing, apoptosis sensitive chronically activated peripheral CD4+ T cells (Dhein *et al*, 1995) and CD8+ T cells (Zheng *et al*, 1995) (mechanism of feed back control of the T cell proliferation). This feed back control mechanism may lead to a pathologically high rate of lymphocyte apoptosis under certain pathological conditions such as HIV infection (Gougeon, 1995), where an enormous rate of fas ligand production due to the viral proteins gp120 and tat can be detected (Baumler *et al*, 1996).

Influence of retinoids on various forms of T cell death

Vitamin A deficiency has been known for a long time to be accompanied with immune deficiency and susceptibility to a wide range of infectious diseases (Goodman, 1994). In vitamin A deficient animals a marked atrophy of the thymus and the spleen has been observed (West *et al*, 1989). On the other hand, retinoids at high doses are toxic and cause involution of lymphoid organs, in particular of the thymus (Dennert and Lotan, 1978), whereas moderate, subtoxic doses of retinoids have been reported to result in a significant increase in thymus weight, numbers of thymic small lymphocytes (Seifert *et al*, 1981), and in cellularity of lymph nodes (Taub *et al*, 1970). Suggestions have been made that the active metabolites of vitamin A, that mediate its effects on the immune system, are the retinoic acids (Mendelsohn *et al*, 1994; Zhao and Ros, 1995) – all-*trans* RA and 9-*cis* RA – which are ligands for the nuclear retinoic acid receptor family (Mangelsdorf, 1994).

Since apoptosis plays a major role in shaping the T cell repertoire, one way in which retinoids can affect the immune system is to influence various forms of T cell death. Indeed it has been demonstrated that retinoids can enhance the rate of glucorticoid-induced death (Iwata *et al*, 1992; Fésüs *et al*, 1995), and can inhibit the TCR-mediated death of thymocytes and T cell hybridomas (Iwata *et al*, 1992; Yang *et al*, 1993; Fésüs *et al*, 1995) but have no effect on the *p53*-induced death (Fésüs *et al*, 1995). While the exact mechanism of activation-induced death of thymocytes is not known, it has been demonstrated in the case of T cell hybridomas that the mechanism by which retinoids inhibit activation-induced death is inhibition of fasL production (Yang *et al*, 1995a). Based on these observations all-*trans* RA was successfully used in *in vitro* and *in vivo* to inhibit activation-induced T cell death of HIV+ individuals (Yang *et al*, 1995b). It was also shown that this protection concerns mainly the CD4+ T lymphocytes and based on the down regulation of fasL production (Szondy *et al*, unpublished observation). Additionally, retinoids were found to be inducers of apoptosis by themselves (Fésüs *et al*, 1995; Szondy *et al*, 1997). In each of these phenomena 9-*cis* RA has proved to be 10–50 times more effective than all-*trans* RA.

Interestingly most of the previously observed biological effects of retinoids on T cell death can be mimicked by glucocorticoids as well. Glucocorticoids are ligands for the glucocorticoid receptor family, which together with the retinoid receptors, belong to (though different subclasses of) the same nuclear receptor superfamily (Mangelsdorf *et al*, 1995). Glucocorticoids themselves are potent inducers of T cell death (Cohen, 1993; Szondy, 1997), enhance the effect of retinoids (Iwata *et al*, 1992), inhibit activation-induced death of thymocytes (Iseki *et al*, 1991) and prevent apoptosis of T lymphocytes by down regulation of fasL production (Yang *et al*, 1995a). Other members of this receptor superfamily (thyroid hormones or vitamin D) have no effect on apoptosis of T lymphocytes (Iwata *et al*, 1992).

What may be the biological significance of these findings? The concentration of all-*trans* retinoic acid needed to initiate or inhibit certain forms of apoptosis in thymocytes is much higher than its physiological plasma level *in vivo* estimated to be 12 nM (De Leenheer *et al*, 1982; Reichert and Fésüs, 1991; Lehman and Franz, 1996). However, the concentration of it may vary around the cells due to a local retinoid production (Wagner *et al*, 1992). 9-*cis* RA was shown to be generated by isomerisation of all-*trans* RA being in equilibrium with and depending on the concentration of all-*trans* RA (Urbach and Rando, 1994). Since the apoptosis-regulating effect of 9-*cis* retinoic acid occurs at a much lower concentration, in case the circulating all-*trans* retinoic acid is converted to the 9-*cis* ligand in sufficient amount in thymocytes, the RA-mediated cell death is initiated. This may be one of the critical events in the initiation of apoptosis of those $CD4^+CD8^+$ double positive thymocytes which have low-affinity TCR and have not been positively selected or eliminated by the negative selection pathway mediated by high-affinity TCR-self antigen interaction. The large majority of thymocytes die through this so called 'default death pathway' (Fésüs, 1991), but the initiator of apoptosis in these cells has not been clarified yet. Though glucocorticoids certainly accelerate the default pathway of apoptosis both *in vivo* and *in vitro*, this does not necessarily mean that the steroids are the only physiological initiators of the death pathway in these cells. One may speculate that the simultaneous enhancing effect of these two ligands may lower the effective concentration for both ligands to induce apoptosis in a large population of thymocytes.

Glucocorticoids, retinoids and the TCR seem to regulate positive and negative selection of thymocytes in a coordinated manner. Glucocorticoids, which also inhibit TCR-induced cell death, were suggested to be required for the transition from $CD4^-CD8^-$ to $CD4^+CD8^+$ cells and additionally to increase the threshold at which an antigen is recognised as high affinity ligand and initiates negative selection (King *et al*, 1995). At low concentrations of glucocorticoids retinoids proved to be additive in inhibiting TCR-mediated cell death (Iwata *et al*, 1992) suggesting that retinoids and glucocorticoids may simultaneously affect the

number of positively selected thymocytes. Additionally retinoids may also affect the differentiation process since selective overexpression of the RARγ receptor in the T cell compartment can affect the ratio of CD8+ and CD4+ T cells (Pohl *et al*, 1993).

Molecular mechanism of action of retinoic acids

All-*trans* and 9-*cis* RA are formed within most cells and are physiological ligands for the retinoid receptors (RARs, RXRs) (Chambon, 1994). These receptors are ligand dependent transcription factors which bind to specific hormone response element (RARE, RXRE) and transactivate specific target genes (Rastinejad *et al*, 1995). All-*trans* RA and 9-*cis* RA are equipotent in activating RAR, while activation of RXR by all-*trans* RA is 50-fold less than by 9-*cis* retinoic acid (Heyman *et al*, 1992). Though all-*trans* RA does not bind to RXR receptors, the latest observation is explained by conversion of the all-*trans* RA to 9-*cis* RA within the cells (Urbach and Rando, 1994) reaching an intracellular concentration sufficient to activate RXR receptors at high extracellular concentrations of all-*trans* RA. Under experimental conditions retinoic acid receptors function in the form of either RAR/RXR heterodimers or RXR/RXR homodimers in the presence of RAs (Zhang *et al*, 1992). It is, however, still an open question whether RXR/RXR homodimer formation can occur under conditions when RXR is expressed at physiological concentrations in cells, though some experiments suggest that it may be so (McCaffery *et al*, 1993). Additionally, RXR can form heterodimers with various other members of the steroid/thyroid/retinoid receptor family such as thyroid receptor, vitamin D_3 receptor, COUP-TF (Kliewer *et al*, 1992a,b; Yu *et al*, 1991). The presence of RXR in these heterodimers is needed to enhance the cooperative binding of these receptors to the DNA, and the transcriptional activation requires only the presence of the cognate vitamin D_3 receptor, thyroid receptor or RAR ligands, but can be modulated by the simultaneous binding of the RXR ligand (Yu *et al*, 1991). These complex interactions and the existence of multiple retinoic acid nuclear receptors (RARα, β and γ) as well as retinoid X receptors (RXRα, β and γ), differentially expressed in various tissues and cell types, explain the pleiotropic effects of retinoids in practically all types of cells in the mammalian organism.

Though a partial functional redundancy exists between the three types of RAR (Mendelsohn *et al*, 1994; Taneja *et al*, 1995) the time and tissue specific expression of the various RARs during development and the observed malformations in RAR null mice (Lufkin *et al*, 1993; Lohnes *et al*, 1993) suggest that at wild-type expression levels various RAR types exert different regulatory roles. RXRα knock out mice also show various malformations suggesting that RXRα has an important role in the transduction of a retinoid signal during development (Kastner *et al*, 1994). The RXRα and β receptors, however, have a widespread (possibly ubiquitous) expression pattern during mouse development and though RXRγ transcripts appear to have a more restricted distribution, RXRγ null mice are apparently normal (Krezel *et al*, 1996). Additionally, RXR$\alpha^{+/-}$/RXR$\beta^{-/-}$/RXR$\gamma^{-/-}$ mutant mice are viable demonstrating that one copy of RARα is sufficient to perform most of the functions of the RXRs. These data suggest that the specificity of the retinoid signalling pathway is determined by the three types of RARs within the RAR/RXR heterodimers and the presence or costimulation of RXRs may affect these signalling pathways independently of their family types.

Involvement of RAR and RXR receptors in regulating spontaneous death of thymocytes

Thymocytes and T cell hybridoma cells express RARα and RARγ but not RARβ (Meco *et al*, 1994; Szondy *et al*, 1997a). This means that in these cells both RARα/RXR and RARγ/RXR heterodimers may function in the retinoid signal transduction pathway involved in regulation of T cell death. In experiments carried out in our laboratories selective RAR subtype agonists, antagonists and RXR agonists (Table 1) were used to investigate which retinoid receptors mediate retinoid actions (Szondy *et al*, 1997a).

We have previously shown that retinoic acids can induce death in mouse thymocytes (Fésüs *et al*, 1995). 9-*cis* RA was found to be more effective than all-*trans* RA suggesting that RXRs might be involved. Indeed, addition of an RXR selective compound (CD2425) at 0.1 μM concentration shifted the dose curve of the all-*trans* RA to that of the 9-*cis* RA. However, neither physiological concentrations of all-*trans* RA nor the RXR agonist alone were able to induce cell death suggesting that both RARs and RXRs participate in the phenomenon.

Using various RAR subtype selective agonists it was found that neither RARα nor RARβ agonists can induce

Table 1 Binding constants and transactivation properties of retinoids used in this study

	Binding K_d/nM			Transact. EC_{50}/nM	
Compound	**RARα**	**RARβ**	**RARγ**	**RXRα**	**Property**
ATRA	**16**	**7**	3	>1000	Agonist
9-cis-RA	**30**	**11**	**20**	**24**	Agonist
CD 336	**8**	131	450	>1000	Agonist
CD2081	**6**	147	753	>1000	Agonist
CD2314	>3760	**145**	no binding	>1000	Agonist
CD437	6500	2480	**77**	>1000	Agonist
CD666	2240	2300	**68**	>1000	Agonist
CD2019	1100	**26**	**160**	>1000	Agonist
CD2325	1144	1245	**53**	>1000	Agonist
CD2425	>1000	1467	712	**54**	Agonist
CD2503	**6**	964	>1000	>1000	Antagonist
CD2665	>1000	**306**	**110**	>1000	Antagonist

Equilibrium dissociation constants (K_d values) for the interaction of the different retinoids with the three RAR subtypes were determined by competition binding experiments using [^{3}H]-CD367 as radiolabelled reference retinoid, that binds with high affinity to RARα, RARβ, and RARγ (K_d=3.7, 4.1 and 1.5 nM, respectively) but does not transactivate RXR. The assays were performed as previously described (Szondy *et al*, 1997a) using nuclear extracts of COS-7 cells transfected with pSG-5-derived expression vectors for RARα, RARβ or RARγ (provided by Dr. M. Pfahl, La Jolla Cancer Research Foundation, La Jolla, CA). Because no radiolabelled RXR specific ligand for binding studies was available, interaction of the retinoids with this receptor type was assessed by a functional transactivation assay as previously described (Szondy *et al*, 1997a)

cell death. Four types of RARγ agonists tested (Table 1), however, proved to be effective inducers of apoptosis. Additionally, an RARγ antagonist (CD2665) at 3 μM concentration prevented cell death induced by either 9-*cis* RA (1 μM) or by combination of all-*trans* RA (0.1 μM) and an RXR analogue (0.1 μM) proving that the induction of cell death by retinoids is mediated via the RARγ receptor. With nearly equal K_d for RARγ the ability of the RARγ agonists to induce apoptosis was found to be strongly dependent on their specificity to RARγ versus RARα (Table 2). This observation suggests that costimulation of RARα may suppress the RARγ-mediated cell death pathway. Indeed, addition of increasing concentrations of an RARα analogue (CD336) inhibited the RARγ-mediated cell death, while combination of an RARα antagonist (CD2503 0.1 μM) with all-*trans* RA at concentrations where all-*trans* RA alone was not sufficient to initiate apoptosis resulted in cell death induction. These data demonstrate that the action of RARα and RARγ are balanced and no cell death can be observed when they are costimulated by physiological concentrations of all-*trans* RA. If however, RXR is also stimulated by addition of an RXR analogue or in case of 9-*cis* RA the RARγ pathway of cell death is initiated. According to these data the role of the stimulation of the RXR is to facilitate the RARγ-mediated cell death pathway (Figure 1).

Table 2 Ligand selectivity and apoptotic potential

Compound	K_d(RARα)/K_d(RARγ)	EC_{10}/nM*
All-*trans*-RA	5	1400
CD2019	7	4540
CD2325	22	1370
CD666	33	193
CD437	84	6.3

*Concentration leading to 10% DNA fragmentation above the baseline level (controls)

Involvement of various RAR and RXR receptors in regulating TCR-mediated death of T lymphocytes

TCR-mediated death of both thymocytes and T cell hybridomas were shown to be inhibited by increasing concentrations of all-*trans* and 9-*cis* RA (Iwata *et al*, 1992; Yang *et al*, 1993; Fésüs *et al*, 1995). 9-*cis* RA was found to be 10 times more potent suggesting again that RXR receptors are involved in the process. Indeed, studies, carried out on T cell hybridomas transfected with cDNA encoding RXRβ, have shown that cells that overexpressed RXRβ were more sensitive to 9-cis RA rescue from TCR-mediated death. In contrast, cells expressing the dominant-negative RXRβ could not be rescued from death with 9-*cis* RA (Yang *et al*, 1995c). Addtionally in wild-type cells, an RAR-selective synthetic ligand had little effect, while simultaneous addition of an RXR selective agonist prevented TCR-mediated apoptosis (Bissonette *et al*, 1995; Yang *et al*, 1995c). Since nur77 which is also a member of the steroid/thyroid/retinoid nuclear receptor superfamily (Mangelsdorf *et al*, 1995) was shown to be involved in the signal transduction of TCR-mediated death (Liu *et al*, 1994), suggestions were made that nur77 functions in the monomer form to upregulate fasL production, while RXR upon ligand binding forms a heterodimer with it and inhibits its action (Okabe and Nawata, 1996). However, selective RXR analogues alone inhibit TCR-mediated death only at concentrations much higher than that required for 9-*cis* RA. Simultaneous addition of the RAR- and RXR-selective retinoids, on the other hand, completely prevent activation-induced apoptosis at concentrations where either alone had relatively little protective effect (Bissonette *et al*, 1995; Yang *et al*, 1995c). These data suggest that binding of not only RXRs but also RARs are required for efficient inhibition of TCR-mediated death.

In similar experiments described previously (Szondy *et al*, 1997a) we have found that, in agreement with the expression pattern, an RARβ-selective compound cannot

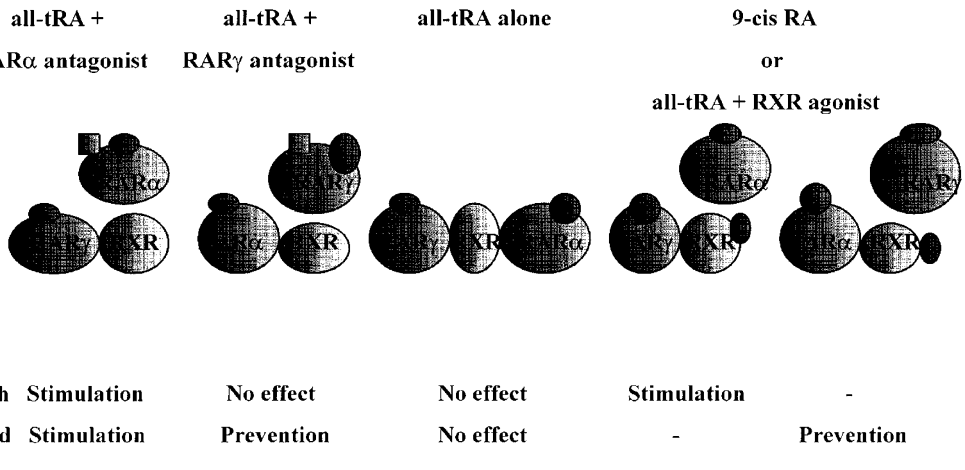

Figure 1 Possible model for the action of RARs and RXRs in regulating various apoptosis pathways of thymocytes. Circles represent binding of agonists while squares binding of antagonists. All-*trans* retinoic acid is at physiological concentrations. Please, note that RXR costimulation by 9-*cis* RA results in different outcome in the two cell death pathways: 1, induction of apoptosis by suspending the balance between the stimulation of the two RARs via facilitating the RARγ pathway; 2, prevention of activation-induced death by suspending the balance between the stimulation of the two RARs via facilitating the RARα pathway

inhibit activation-induced apoptosis in thymocytes and T cell hybridomas. RARγ-selective compounds (CD437, CD2325 and CD2019 with an EC_{10} value of 6.3 nM, 1.3 μM and 4.5 μM, respectively), on the other hand, stimulated and did not inhibit TCR-mediated death. Two RARα-selective compounds (CD336 and CD2081) at a concentration of 0.1 μM, however, efficiently inhibited TCR-mediated death (unpublished observations). Their inhibitory effect corresponded well to their specificity for the RARα receptor and to the downregulation of fasL production in a T cell hybridoma cell line (unpublished observations), suggesting that RARα alone might be involved in blocking death. Indeed, an RARα antagonist (CD2503) at 1 μM concentration prevented inhibition of TCR-mediated death achieved by addition of either 9-*cis* RA (1 μM) alone or a combination of all-*trans* RA (0.1 μM) and an RXR analogue (CD2425 0.1 μM).

Why do RAR panagonists (Yang *et al*, 1995c) or low (physiological) concentrations of all-*trans* RA not prevent the TCR-mediated death when they are also potent RARα activators? Since the RARα specific compounds used in our study and all-*trans* RA have similar K_d for the RARα, the only explanation for the lack of the inhibitory effect is that the panagonist all-*trans* RA costimulates RARγ as well. Indeed, addition of an RARγ antagonist (CD2665) in 0.1 μM concentration shifted the inhibitory dose curve of all-*trans* RA to that of the 9-*cis* RA (unpublished observation). Furthermore, increasing concentrations of the RARγ analogue (CD437) prevented inhibition of activation-induced death by the RARα analogue (unpublished observation). These data suggest that when costimulated (in case of physiological concentration of all-*trans* RA) the apoptosis stimulatory action of RARγ and the apoptosis inhibitory action of RARα are balanced and no change in the cell death rate can be observed. Since costimulation of RXR receptors makes both all-*trans* RA and a panangonist RAR analogue (Bissonette *et al*, 1995; Yang *et al*, 1995c) effective apoptosis inhibitors, we suggest that the role of RXR costimulation in the inhibition of TCR-mediated death is to facilitate the RARα-mediated inhibitory pathway (Figure 1).

Possible molecular explanation of the effects of RARα or RXR costimulation on the apoptosis machinery

It has already been demonstrated that RXRs are not always passive participants within the various transcription factor heterodimers in regulation of transciption. In some cases both the specific ligand and 9-*cis* RA can turn on transcription if, however, both ligands are present the transcription rate is enhanced (Mekherjee *et al*, 1997). In some other cases additional binding of 9-*cis* RA inhibits transcription (Haussler *et al*, 1995). In the case of inhibitory thyroid hormone response elements, binding of 9-*cis* RA releases RXR from the thyroid receptor/RXR heterodimers and suspends thyroid mediated inhibition of transcription (Cohen *et al*, 1995). Our data are the first to show that activation of RXRs by 9-*cis* RA or other ligands can regulate antagonistic RARα and RARγ-mediated processes.

Our studies are not sufficiently detailed to decide the precise mechanism by which stimulation of RARα leads to the observed inhibition of RARγ-mediated effects. One possibility is that RARα acts downstream of RA-RARγ binding initiating various anti-apoptotic processes. Alternatively both RARα and RARγ may regulate the transcription of specific proteins responsible for initiation of apoptosis. The specific proteins responsible for initiation of the RARγ-mediated or TCR-induced death of thymocytes have not been determined yet. Since, however, those RARγ analogues which have higher affinity for RARα require higher concentrations to induce the same rate of apoptosis or to stimulate the TCR-mediated apoptosis, one possibility is that the co-stimulated RARα may compete with RARγ for RXR binding or costimulated RARα/RXR heterodimers may compete with RARγ/RXR heterodimers for DNA binding or transactivation sites. Stimulation of RXR in the same setting might facilitate binding of one or the other RAR receptor to the RXR receptor or binding of the RAR/RXR heterodimer to the DNA. Interestingly, RXR costimulation results in a different outcome in the two cell death pathways: in apoptosis induction the RARγ occurs while in inhibition of TCR-mediated death the RARα pathway is activated. This may suggest that other transcription factors or retinoid receptor phosphorylation induced by the TCR signalling pathway are also involved in the retinoid receptor interplay.

From this point of view it is worth noting that the expression of tissue transglutaminase, an effector element of apoptosis (Fésüs *et al*, 1987; 1996; Piacentini *et al*, 1994) was increased in thymocytes if apoptosis was induced via either the RARγ (Szondy *et al*, 1997a) or the TCR-mediated pathway (Szondy *et al*, 1997b) *in vivo* and was not if RARα was costimulated. The promoter of this protein contains a 30-base pair retinoid response element (mTGRARE1) which consists of three hexanucleotide half-sites in DR7/DR5 motif. This response element binds both RAR/RXR heterodimers and RXR homodimers, can be partially activated by RAR or RXR agonists, but for the full activation the panagonist 9-cis RA is required (Nagy *et al*, 1996). It is conceivable to believe that genes with such regulatory elements may be targets of the complex effects of retinoids described above.

Perspectives

Our findings suggest that both the type of retinoids available and fine tuning of RAR expression leading to various RARα/RARγ activation ratios in a thymocyte cell population may be determining factors for a cell to stay alive or to die.

The tissue distribution of the RARγ transcript suggests a role for this receptor in morphogenesis, chondrogenesis, and differentiation of squamous epithelia (Lohnes *et al*, 1993). Null mutant mice of all RARγ isoforms exhibit growth deficiency, early lethality, various forms of embryonic malformation and squamous metaplasia at ectopic locations (Lohnes *et al*, 1993); several of the observed phenotypic changes may be explained by the perturbation of programmed cell death during development. RARγ together with RARα plays a critical role in maintaining keratinocyte differentiation and cornification (Saitou *et al*, 1995); cornification and apoptosis are closely linked phenomena and both may occur and be regulated by

retinoids, perhaps through RARγ, in the skin (Polakowsaka, 1994). These data suggest that the physiological importance of retinoid-induced apoptosis through RARγ may not be restricted to the thymus and the immune system. Furthermore, there are significant therapeutic implications of the existence of a well-characterised, retinoid-initiated apoptosis pathway. If the presence of RARγ in a cell type renders it susceptible to apoptosis (Marshall *et al*, 1995; Fanjul *et al*, 1996; Melino *et al*, 1997a,b) cell death will be initiated by addition of RARγ-selective compounds. If the γ receptor is expressed in malignant or autoreactive cell populations (or introduced into such population by gene transfer) these cells might be eliminated by apoptosis as a part of a new therapeutic strategy against cancer or autoimmune diseases. In this context it is worth noting that recently new retinoid structures were identified which were highly effective against lung cancer cells and various other tumours via inducing apoptosis (Lu *et al*, 1997). Interestingly, all the effective compounds bound preferentially to RARγ, providing additional evidence for a more general importance of the RARγ mediated cell death pathway. Furthermore, our data also suggest the RARγ apoptosis pathway can be potentiated by the administration of either RARα-antagonists or RXR agonists thus providing the basis of appropriately balanced and perhaps cell type specific therapeutic protocols for retinoids.

Pathologically high level of fasL production has been suggested to mediate apoptosis of lymphocytes in HIV+ individuals (Gougeon, 1995), cell death of hepatocytes in acute viral hepatitis or to provide protection against cytotoxic T lymphocytes by some tumours. In these cases a possible down regulation of fasL production by RARα selective compounds could prevent pathological rate of apoptosis and elimination of tumour killing lymphocytes. These data suggest that selective retinoid compounds may be a powerful therapeutics in the near future via regulating various cell death pathways.

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