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Apo2L/TRAIL and immune regulation

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1. ABSTRACT

Apo2L/TRAIL is a member of the TNF family, with its receptors DR4 and DR5 containing a death domain. Multiple tumors are sensitive to Apo2L/TRAIL-induced apoptosis, while normal cells are not, so it constitutes a promising new antitumoral therapy. In this review we deal rather with the physiological role of Apo2L/TRAIL, which, in one hand, is clearly related with immune antitumoral surveillance. However, a role of Apo2L/TRAIL as a fine-tuning regulator of the immune system, especially in the regulation of $CD8^+$ T cell activation and memory, has been also demonstrated. In fact, Apo2L/TRAIL can be considered as an additional mechanism needed to prevent the development of autoimmune disease. Indeed, recent developments indicate that Apo2L/TRAIL can be also useful as a treatment against certain chronic autoimmune diseases.

2. INTRODUCTION

2.1. Apo2L/TRAIL and its receptors

After TNF and Fas ligand (FasL), Apo2 ligand/TNF-related apoptosis-inducing ligand (TRAIL) was the third member of the TNF family to be cloned. Apo2L/TRAIL was cloned and characterized almost simultaneously by two groups, showing that it could induce apoptosis in a variety of tumoral cells in a Fas-independent fashion (1, 2). As FasL, Apo2L/TRAIL is a type II membrane glycoprotein, with a predicted MW of its polypeptide moiety of 32.5 kDa. However, the MW of the mature, fully glycosylated form of the protein is of 41 kDa. The soluble form of the protein after metalloproteinase-mediated cleavage exhibits a MW of 24 kDa. Several receptors for Apo2L/TRAIL, termed DR4 (3), DR5 (4, 5), and the decoy receptors DcR1 and DcR2 (4, 5), have been cloned. Only DR4 and DR5 posses a death domain in their

cytoplasmic tail and have pro-apoptotic potential, while the decoy receptors down-modulate the activity of the former receptors by sequestration of the bioactive ligand. Recent studies demonstrated that it is DR5 the receptor with a higher pro-apoptotic potential, at least in tumoral cells (6). The caspase-dependent apoptotic pathway initiated by ligation of death receptors is well characterized (7).

2.2. Apo2L/TRAIL as an antitumoral agent

The low toxicity of recombinant versions of Apo2L/TRAIL or of anti-DR4 or DR5 mAbs on normal cells, while exerting a potent pro-apoptotic activity on a variety of human tumors, has made possible to develop these agents as new and promising antitumoral therapies (revised in(8-10)).

Indeed, one of the physiological roles of Apo2L/TRAIL seems to be immune antitumoral surveillance. The possibility to exert cytotoxicity against tumoral cells through Apo2L/TRAIL has been demonstrated for activated CD4+ T cells (11-13), NK cells (14) and monocytes (15). More recent data using TRAIL knockout mice have shown that these mice are more susceptible to tumor initiation by carcinogens and to metastasis dissemination than wild type mice (16), with spontaneous haematological tumors appearing at old age (17).

3. IMMUNE TOLERANCE IN THE T CELL COMPARTMENT

3.1. Central and peripheral tolerance

Immune tolerance is a complex process, necessary to maintain normal homeostasis and to avoid autoimmunity. Regarding T cells, central tolerance is achieved during thymic maturation, mainly by deletion of autoreactive immature thymocytes (negative selection) (18). However, complete T cell tolerance is also dependent on peripheral tolerance mechanisms, acting on mature T cells that have reached the periphery (19). Several mechanisms account for the achievement of T cell peripheral tolerance, and defects in just one of them are normally associated with autoimmunity. Among these mechanisms are: i) the induction of anergy through antigen presentation by non-antigen presenting cells (APC), in the absence of costimulation (20), or by immature APCs (21); ii) the action of regulatory T cells of $CD4^+CD25^+$ phenotype (22); and iii) the termination of T cell immune responses (23).

The regulated termination of T cell immune responses seems to be dependent in turn on several complex, possibly overlapping, cellular and molecular mechanisms. On one hand, T cell activation results in induction of the expression of the negative regulator CTLA-4, which competes with CD80/CD86 for the T cell co-stimulator CD28 (24). On the other hand, it is clear that deprivation of immuno-stimulatory cytokines such as IL-7, IL-2 and IL-15 as a consequence of antigen exhaustion is one of the main causes of down-modulation of T cell responses (25). It has been suggested that this is not just a passive cell death process, but that is related with the activated status of the expanded T cell population, being re-termed as "activated T cell autonomous cell death" (26). The BH3-only pro-apoptotic member of the Bcl-2 family Bim has been demonstrated to play a main role in this process, and defects in its expression are associated with autoimmunity (27, 28).

3.2. Activation-induced cell death

Finally, activation-induced cell death (AICD) of T cells generated by clonal expansion is also implicated in normal termination of immune responses. This process was first reported to be dependent on death receptor/death ligand interplay, especially on the Fas/Fas ligand (FasL) system (29, 30). In fact, *lpr* and *gld* mice, deficient respectively in functional Fas or Fas ligand expression (31, 32), or humans with similar defects (33), have systemic autoimmune disease characterized by lymphoproliferation.

Naïve T lymphocytes are not sensitive to death receptor-induced apoptosis neither to AICD, and T cell blasts begin to be sensitive to AICD induction only at day-6 (34, 35). This sensitizing to AICD and to death receptor-induced apoptosis is related with the expression or regulation of pro- or anti-apoptotic proteins during the process of T cell blast generation. Our group has recently characterized using human T cell blast generation that the higher sensitivity of normal human T cell blasts to apoptosis and AICD as compared with naïve T cells correlates with the disappearance of c-$FLIP_S$ expression, which is readily induced after 1 day of activation of fresh PBL, and with the increased expression of Bcl-x_S and Bim (36). This indicates that T cell blasts are more sensitive than recently activated T cells to death receptor triggering by downmodulation of c-$FLIP_S$, a protein that interferes with signal transduction between death receptors and the activation of the caspase cascade (37) and also to the mitochondrial apoptotic pathway through the upregulation of the pro-apoptotic members of the Bcl-2 family Bcl-x_S and Bim (38, 39). The expression pattern of Bim in human T cells does correlate with its proposed role in the termination of T cell responses demonstrated previously in mice models (28, 40). Also in agreement with these results, recent studies have shown the same pattern of c-$FLIP_S$ expression during normal human T cell blast generation (41), and the increase in Bim levels after TCR triggering in human long-term T cell lines (42). We have also demonstrated that this pattern of expression of relevant pro- and anti-apoptotic proteins between naïve T cells and T cell blasts is similar in both $CD4^+$ and $CD8^+$ T cell subpopulations (43).

4. ROLE OF APO2L/TRAIL IN THE AICD OF HUMAN T CELLS

4.1. First indications

The possible implication of Apo2L/TRAIL in AICD was first suggested by Marsters *et al.*, who showed that recombinant Apo2L/TRAIL induced a moderate amount of cell death in peripheral blood T cells, but only after culture in the presence of IL-2 (44). Our group showed shortly afterwards that Apo2L/TRAIL was expressed by fresh human PBL, but that its expression clearly augmented during the process of T cell blast generation. We also

showed that upon re-stimulation of human T cell blasts, bioactive Apo2L/TRAIL was secreted to the supernantant, being cytotoxic against the human T cell leukemia Jurkat, further suggesting its possible implication in the AICD of human T cells (34).

4.2. Role of CD59 in AICD. Relationship with Apo2L/TRAIL

Upon PHA or anti-CD3 mAb re-stimulation, both bioactive FasL and Apo2L/TRAIL were secreted by human T cell blasts (34), but we observed that upon re-stimulation with mAb directed against CD59, bioactiveApo2L/TRAIL was released in the absence of FasL secretion (45). CD59 is a glycosylphosphatidylinositol (GPI)-anchored glycoprotein from the Ly-6 superfamily, which acts as endogenous inhibitor of complement-mediated lysis through binding to the complement components C8 and C9 (46). This GPI-linked molecule, like its murine homologue Thy-1, also transduces an accessory signal for T cell proliferation and stimulates IL-2 synthesis through the TCR□/ZAP-70 signalling cascade (47). Our data suggested the intriguing possibility that CD59, while inhibiting complement attack, would also contribute to T cell activation and to down-modulation of T cell responses. In fact, it has been reported that mice deficient in GPI-linked proteins have autoimmunity problems (48).

5. IN WHICH MOLECULAR FORM IS BIOACTIVE APO2L/TRAIL RELEASED FROM HUMAN T CELL BLASTS?

5.1. FasL and Apo2L/TRAIL are secreted associated with exosomes during AICD of human T cell blasts

In posterior studies, our group was interested in the characterization of the molecular form in which FasL and Apo2L/TRAIL were released from re-stimulated human T cell blasts to maintain thier bioactivity intact. Although it was initially reported that the soluble form of FasL, generated through the metalloproteinase-mediated cleavage of the membrane protein, retained its cytotoxic potential (49, 50), later studies demonstrated that membrane-bound FasL is the functional form of the molecule, being the toxic activity of the cleaved soluble form much lower (51-53). In fact, it was proposed that FasL proteolytic processing was a mechanism of functional downregulation (53).

In a subsequent work, we characterized that bioactive FasL and Apo2L/TRAIL were rapidly released to the supernatant of human T cell blasts undergoing AICD in the form of whole, non-proteolyzed proteins, associated with a particulate, ultracentrifugable fraction. This fraction was characterized by scanning electron microscopy as microvesicles/exosomes of 100 to 200 nm of diameter and it was also developed a flow cytometry technique to evaluate FasL and Apo2L/TRAIL expression on the surface of these secreted exosomes. In this way, both death ligands are secreted in their membrane-associated form, maintaining their bioactivity. (54).

5.2. Intracytoplasmic storage compartments for FasL and Apo2L/TRAIL in human T cells blasts

We further characterized the nature of the cytoplasmic compartments where FasL and Apo2L/TRAIL were stored inside human T cell blasts, and found that they were similar to those described for the storage of exosomes with antigen-presenting function in APC (55). The exosomes described in APC are 60-100 nm in size and express high amounts of antigen-loaded MHC-II, which are secreted by professional APC, such as B and dendritic cells. Exosomes are stored in APC in a post-Golgi, pre-lysosomal compartment with the structure of a multivesicular body (MVB), and contain membrane proteins correctly oriented, suggesting that the internal vesicles are formed by inward vesiculation of the limiting membrane of the organelle (55).

We showed by confocal microscopy that, in human T cell blasts, FasL and Apo2L/TRAIL co-localized with lamp-1, CD63 and hsc-73 (56), proteins localized respectively in the outer membrane or in the internal membranes of the MVB, or inside the exosomes of APC (57, 58). Confocal microscopy and immuno-electron microscopy (IEM) analysis revealed that most of FasL and Apo2L/TRAIL were stored in the interior of the same MVB in T cell blasts, with PHA re-stimulation inducing the release of exosomes containing both FasL and Apo2L/TRAIL, while exclusive CD59 triggering resulting in the specific release of Apo2L/TRAIL-containing exosomes. The observation by IEM of membrane compartments with exclusive Apo2L/TRAIL labelling close to MVB containing both FasL and Apo2L/TRAIL offered the first clue to understand the differential secretion of FasL or Apo2L/TRAIL upon different surface triggering. The physical proximity of these compartments and even the contact between their membranes could suggest that they are the result of the maturation of a common post-Golgi compartment (56). We also showed that shortly after re-stimulation, the MVB compartments migrated towards the membrane of the cell, where the external membrane of the compartment finally fused with the plasma membrane, leaving a portion of the internal exosomes to be secreted intact, loaded with correctly oriented, bioactive FasL and/or Apo2L/TRAIL (56). All these considerations are summarized in a schematic way in Figure 1.

5.3. Differential sorting of FasL and Apo2L/TRAIL

The differential sorting of FasL and Apo2L, belonging to the same family of death messengers, should be adscribed to differences in their intracytoplasmic domains. The polyproline sequences of the FasL cytoplasmic tail was mapped as the domain responsible for sorting to secretory lysosomes in human hematopoietic cells (59), and more recently, the SH3-containing adaptor responsible for its targeting has been characterized as the proline, serine, threonine phosphatase-interacting protein (PSTPIP; (60)). However, these polyproline sequences are absent in the short Apo2L/TRAIL cytoplasmic tail. Typically, the sorting of membrane proteins to lysosomal-like compartments is dependent on the interaction of the AP3 adaptor with dileucine or tyrosine motifs, in a clathrin-

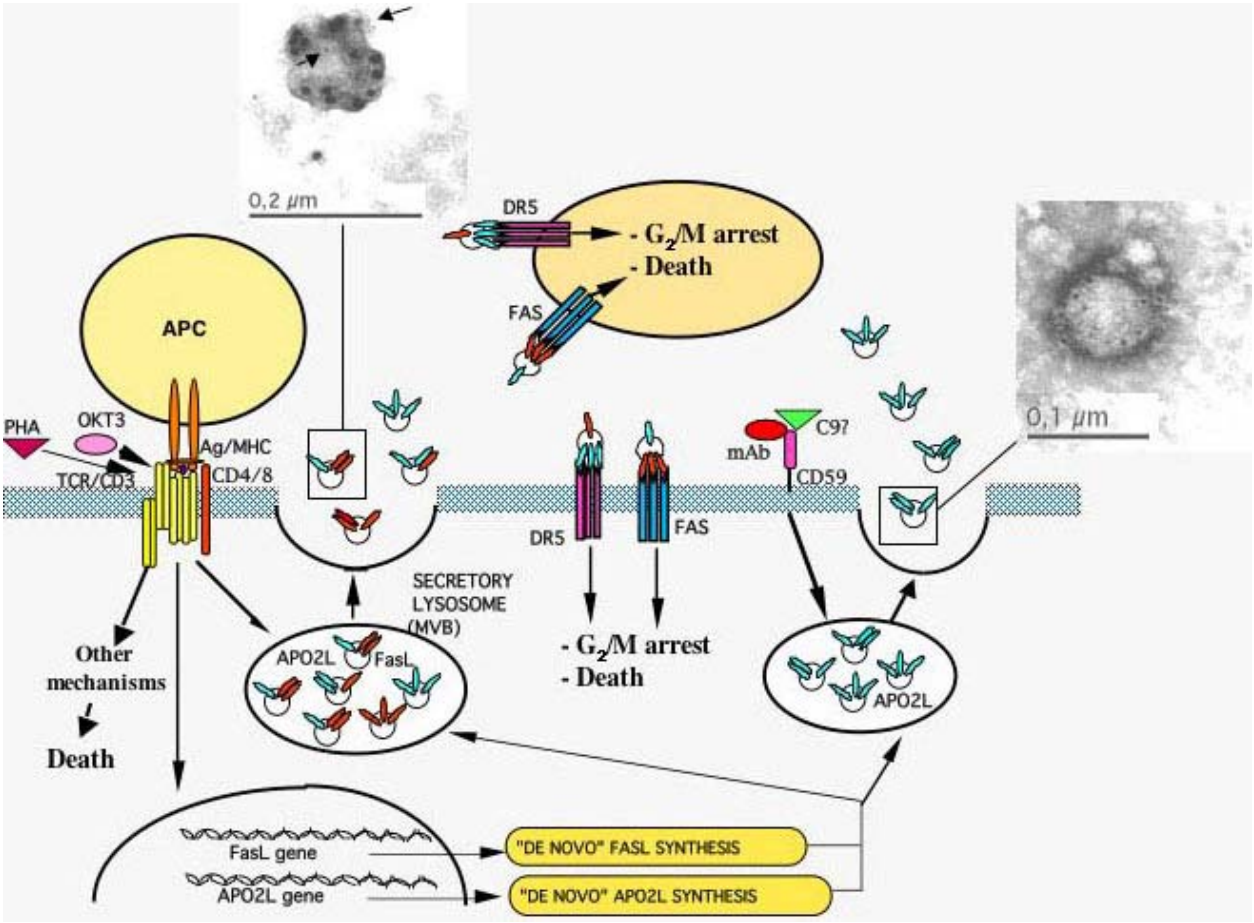

Figure 1. Apo2L/TRAIL and FasL are secreted associated with exosomes during human T cell blast AICD. The events occurring during the re-stimulation of human T cell blasts through the TCR/CD3 or through CD59 are depicted in the scheme. FasL and/or Apo2L/TRAIL-carrying exosomes could act in an autocrine or paracrine manner. Ligation of DR5 or Fas would induce cell death if T cell blasts had been re-stimulated, or rather G_2/M arrest if they had not. The insets correspond to immuno-electron microscopy of exosomes secreted from human T cell blasts after re-stimulation with PHA (left inset) or with anti-CD59 mAb (right inset). FasL is detected as 15 nm gold particles and Apo2L/TRAIL as 5 nm gold particles. The arrows in the left inset correspond to the detection of two Apo2L/TRAIL dots. See additional details in the text.

independent mechanism (61). While no tyrosine residue is present in the Apo2L/TRAIL cytoplasmic tail, a Leu-Gly sequence is localized immediately after its transmembrane domain. Clearly, more studies are needed to characterize both the sorting and the signal transduction pathways that trigger the differential release of both molecules in T cell blasts.

5.4. Surface expression of Apo2L/TRAIL in activated human T cells

In those studies, we detected very low surface expression of Apo2L/TRAIL in human T cell blasts, before of after re-stimulation, indicating that it is mainly stored in the described cytoplasmic compartments before re-stimulation, and mainly secreted to the supernatant associated with exosomes after re-stimulation (54, 56). In comparable cell populations, other groups have described similar results, with the additional observation that Apo2L/TRAIL expression could only be detected on the surface of activated T cells or monocytes after exposure to IFN-alpha or IFN-beta (11, 62, 63).

6. FINE TUNING OF THE IMMUNE RESPONSE BY APO2L/TRAIL

6.1. Apo2L/TRAIL inhibits IL-2-dependent human $CD8^+$ T cell blast growth

In the studies described, supernatants obtained from normal human T cell blasts were tested for apoptosis induction using a functional bioassay on tumoral Jurkat T cells, which are constitutively sensitive to FasL and to Apo2L/TRAIL-induced cell death. However, as indicated above, naïve T lymphocytes are not sensitive to death receptor-induced apoptosis neither to AICD, and they begin to be sensitive only at the T cell blast stage. In order to give physiological validity to our previous observations, we undertook a systematic study using normal human T cell blasts from healthy donors to characterize the effect of

cytotoxic anti-Fas mAb, of rApo2L/TRAIL, or of supernatants containing bioactive FasL and/or Apo2L/TRAIL associated with exosomes, on normal human T cell blasts, using mixed T cell blasts populations (36), and also separating $CD4^+$ and $CD8^+$ T cell blasts (43, 64). In these experiments, fresh PBL were activated by a pulse of PHA and then they were cultured for 6 days in the presence of 30 IU/ml of exogenous IL-2, arriving to the T cell blast stage. Then, T cell blasts were treated as described above in 24 or 48h assays, which were performed in the presence or in the absence of exogenous IL-2.

In the absence of IL-2, T cell blasts begin to die at 24h and cell death was prominent after 48h (36), especially in $CD8^+$ T cell blast (43). In these conditions, no additional effect of death receptor ligation could be observed in the mixed T cell blast population (36), in agreement with previous results from other groups (65). Hence, the mechanism of down-modulation of T cell responses dependent on cytokine deprivation, and mainly affecting $CD8^+$ T cell blast regulation, seems to predominate on death receptor-induced apoptosis (43).

In the presence of IL-2, and in the absence of any pharmacological treatment, cytotoxic anti-Fas mAb or rApo2/TRAIL induced rather inhibition of IL2-dependent growth and not cell death on normal human T cell blasts. This observation was new and rather unexpected. However, T cell clonal expansion shutdown by FasL and/or Apo2L/TRAIL would be enough to justify their immuno-regulatory role, in a process that seems independent of apoptosis induction (36). The effect of rApo2L/TRAIL was always less potent than that of Fas ligation when analyzed in the mixed T cell population. However, when analyzing $CD4^+$ and $CD8^+$ T cell blasts separatedly, it was observed that rApo2L/TRAIL exerted inhibition of IL-2-dependent T cell growth preferentially on $CD8^+$ T cell blasts, contrary to that observed for Fas ligation, which exerted its effect similarly on both T cell subsets. Death receptor ligation on $CD4^+$ or $CD8^+$ T cell blasts induced to different extents cell cycle arrest in G_2/M (64). Previous studies have already described inhibition of growth in the absence of apoptosis induction by rApo2L/TRAIL on activated murine T cells or in long-term auto-reactive human $CD4^+$ T cell lines (66-68). In the report by Lünemann *et al.*, it was shown that rApo2L/TRAIL induced a decrease in CDK4 expression, and the authors concluded that, in consequence, rApo2L/TRAIL should induce cell cycle arrest at the G_1/S transition (68). However, our studies were not directly comparable, since our assays were performed in the presence of IL-2 and the results were completely dependent on the presence of the cytokine. Our results indicate that rApo2L/TRAIL on $CD8^+$ T cell blasts and Fas ligation on both $CD4^+$ and $CD8^+$ T cell blasts inhibit IL-2-dependent cell growth by cell cycle arrest in G_2/M. The connection between death receptor ligation and cell cycle arrest in G_2/M in the absence of apoptosis induction is not known. Several p53-dependent mechanisms have been described for cell cycle arrest in G_2/M in response to genotoxic stress (69). However, p53-independent and PARP-dependent mechanisms have been also described in tumor cells, which result in the same type of cell cycle arrest (70). Regarding Apo2L/TRAIL, the effect could be also mediated by its ability to transduce non-apoptotic signals, dependent on the activation of the NF-kappa B, JNK and p38 MAPK pathways, recently characterized in detail (71, 72). On the other hand, the G_2/M arrest observed could represent a first step towards slowly entering apoptosis. Clearly, further studies are needed to clarify this new immuno-regulatory mechanism, in a process that seems, at least initially, independent of apoptosis induction.

6.2. Physiological validation

The physiological validity of the observations made with anti-Fas mAb or with rApo2L/TRAIL were confirmed by the demonstration of FasL and Apo2L/TRAIL intracellular expression in human $CD4^+$ or $CD8^+$ T cell blasts and their secretion in their bioactive form upon PHA, anti-CD3 or anti-CD59 mAb activation. As expected from our previous results using mixed T cell blast populations, PHA or anti-CD3 activation of separated $CD4^+$ and $CD8^+$ T cell blasts resulted in secretion of FasL and Apo2L/TRAIL, while CD59 ligation resulted preferentially in Apo2L/TRAIL secretion. We also observed using a bioassay on Jurkat cells that although $CD8^+$ T cell blasts were more sensitive to Apo2L/TRAIL regulation, $CD4^+$ T cell blasts were able to secrete higher amounts of bioactive Apo2L/TRAIL upon PHA stimulation. This could mean that regulation could proceed in trans between both T cell subsets. In addition, we showed that direct CD3 or CD59 ligation in the presence of IL-2 was associated with the inhibition of normal T cell blast growth in the absence of cell death induction, in agreement with the effect of the cytotoxic anti-Fas mAb CH11 or of rApo2L/TRAIL (36, 64). These data defined precisely the role of Apo2L/TRAIL in normal human T cell regulation, and signaled death receptor-induced inhibition of IL2-dependent T cell growth as a new immuno-regulatory mechanism (36, 64).

6.3. Re-stimulation through CD3 or CD59 sensitizes T cell blasts to Apo2L/TRAIL-induced apoptosis

On the other hand, it has been clearly shown that if T cell blasts are re-stimulated, they become sensitive to Fas-induced apoptosis (73, 74). In the study of Muppidi and Siegel, the sensitizing effect of CD3 ligation on Fas-induced apoptosis was attributed to Fas recruitment to lipid rafts. In agreement with this, we observed that a pulse through CD3 or CD59 in the presence of IL-2 sensitized human T cell blasts to subsequent Fas or Apo2L-induced cell death, also inducing a reduction in c-$FLIP_L$ and c-$FLIP_S$ levels (36).

In fact, the described need of re-stimulation for T cell blasts to become sensitive to death receptor-induced apoptosis has led to the proposal that death receptors cannot be implicated in the down-modulation of T cell responses against infection, since this process takes place after antigen exhaustion (74). However, the inhibition of IL-2-dependent T cell blast growth by death receptor ligation does not require re-stimulation and would be enough to justify the immuno-regulatory role of FasL or Apo2L/TRAIL (36, 64).

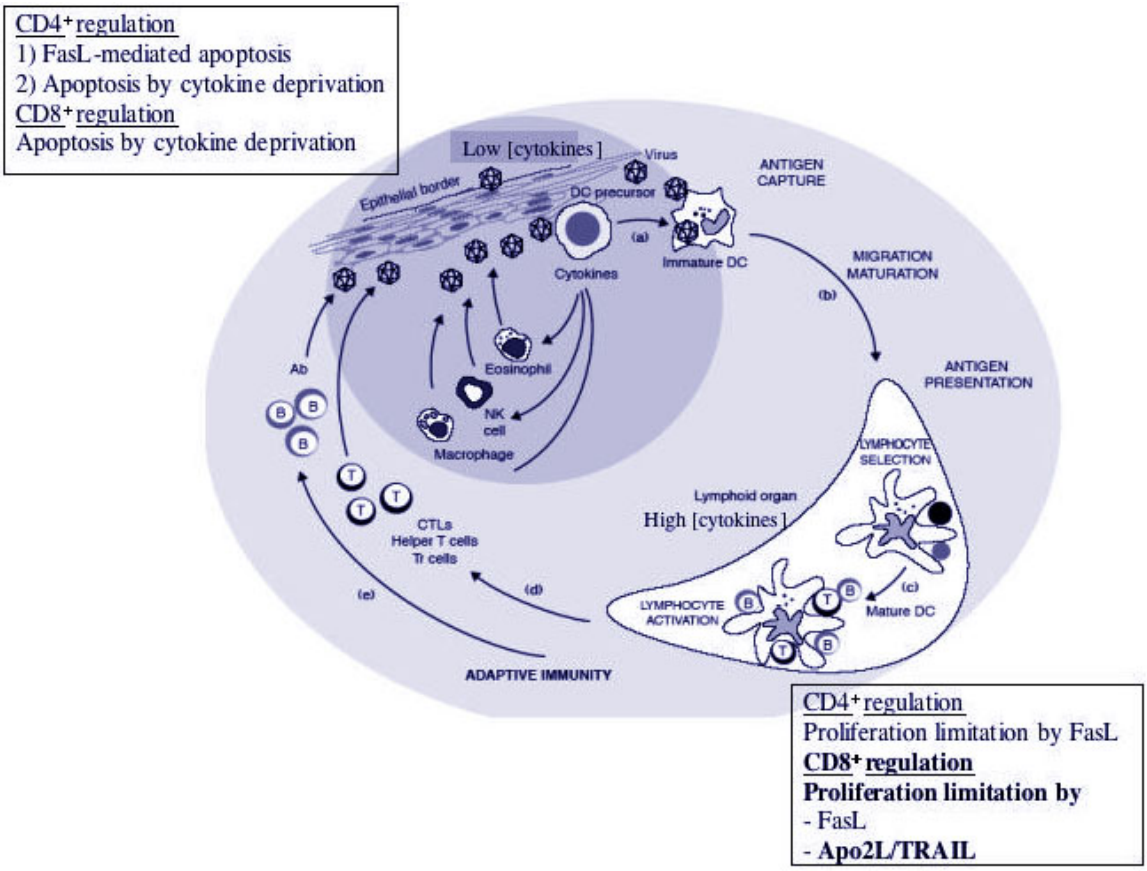

Figure 2. A model for the regulation of T cell activation by Apo2L/TRAIL and FasL. See details in the text. (Modified with permission from an original Figure in reference 93).

6.4. A model for the regulation of human T cell activation

All these data allow us to propose a model in which down-modulation of normal human T cell activation would take place in two different scenarios, depending on the amount of cytokines that stimulate T cell growth. It could be considered that regulation in the presence of high amounts of cytokines would take place in peripheral lymphoid organs during a response against infection, while the contrary would occur at the sites of infection, after migration of effector cells. In the first case, local expression of FasL would inhibit the growth of both T cell subsets, while local expression of Apo2L/TRAIL would inhibit preferentially the growth of $CD8^+$ T cell blasts. This regulation seems to take place in both cases by cell cycle arrest in G_2/M, by a still unsolved mechanism (64). However, once at the site of infection, and in the absence of high amounts of cytokines, down-modulation of the response, and especially that of human $CD8^+$ T cell blasts, would take place rather by cytokine deprivation (43). All these considerations are summarized in schematic form in Figure 2 in the context of a viral infection.

6.5. A role of Apo2L/TRAIL in regulating $CD8^+$ T cell memory

The group of Dr. Schoenberger described recently using murine models and *in vivo* experiments another scenario in which $CD8^+$ T cell expansion is also regulated by Apo2L/TRAIL. In normal conditions, $CD8^+$ T cell expansion proceeds in the presence of $CD4^+$ T cell help, through the secretion of stimulatory cytokines or through the delivery of maturation signals to dendritic cells. One of the consequences of this primary T cell expansion is the generation of memory $CD8^+$ T cells, which are able to undergo a second round of clonal expansion upon re-stimulaton, for which they no longer need $CD4^+$ T cell help (75). However, if the initial clonal expansion takes place in the absence of $CD4^+$ T cell help, memory $CD8^+$ T cells do not undergo the second round of clonal expansion, because they all die by Apo2L/TRAIL-mediated AICD during the re-stimulation (76). The same group has described recently a similar situation during the homeostatic T cell proliferation that takes place during T cell adoptive transfer in lymphopenic mice, which also generates "memory-like" $CD8^+$ T cells (77).

6.6. A possible role for Apo2L/TRAIL in the regulation of B cell activation

Regarding the regulation of B cell activation, at least one study has suggested that plasma cell AICD could be mediated by endogenous Apo2L/TRAIL (78). However, not much more is known about the regulation of plasma cell activation or the termination of B cell responses.

6.7. Apo2L/TRAIL implication in tumor counterattack against T cell effectors?

It has been reported that a variety of malignant tumors show increased expression of FasL, evading immune control via induction of apoptosis of effector immune cells, a mechanism termed as the "tumor counterattack hypothesis" (79). However, the lack of expression of surface FasL by the same tumors and their consequent inability to kill T lymphocytes has questioned this hypothesis, such in the case of melanoma cells (80). However, other factors could be implicated, such as the balance of soluble versus membrane-bound forms or the secretion of death ligands on the surface of exosomes. Regarding Apo2L/TRAIL expression in tumors, it has been shown at least in one study that adenocarcinomas engineered to express Apo2L/TRAIL elude immune control by T cells (81). Using the human melanoma cell line MelJuso, we showed that these cells expressed preformed FasL and Apo2L/TRAIL, that they secreted them associated with exosomes upon melanoma activation with PHA or with □-melanocyte stimulating hormone, and that the tumor-derived exosomes were toxic against normal human T cell blasts. However, the secretion of bioactive Apo2L/TRAIL was much more limited than that of FasL (82).

7. APPLICATIONS IN AUTOIMMUNE DISEASE

7.1. Data from knockout mice

The role of Apo2L/TRAIL in the regulation of T cell responses has been confirmed in the Apo2L/TRAIL knockout mice, which resulted more susceptible to experimental models of autoimmune disease induction than wild type mice, such as experimental autoimmune encephalomyelitis and collagen-induced arthritis (83). In the same study, it was suggested that the Apo2L/TRAIL knockout mice had also impaired negative selection in the thymus (83). However, this last point was not confirmed in studies from other groups (84). More recently, another group have demonstrated that Apo2/TRAIL receptor knockout mice are defective in the regulation of macrophage activation, producing large amounts of pro-inflammatory cytokines, a scenario also compatible with their greater sensitivity to autoimmune disease, although the authors did not test this possibility (85).

7.2. Apo2L/TRAIL as a guardian against autoimmune disease

The first experimental indications on a possible role of Apo2L/TRAIL to prevent autoimmune disease came in fact from the work of Dr. Chen's group, which demonstrated that mice treated with anti-Apo2L/TRAIL blocking antibodies were more susceptible to the development of collagen-induced arthritis (66) and also to the development of experimental autoimmune encephalomyelitis (67). Another study has related the beneficial effect of IFN-alpha treatment in multiple sclerosis patients to its positive effect on the expression of Apo2L/TRAIL in immune cells (86).

7.3. Apo2L/TRAIL as a treatment for autoimmune disease

Furthermore, two studies performed in experimental animal models of arthritis have used Apo2L/TRAIL gene therapy (87-89). In the study of Yao *et al.*, intra-articullar adenoviral-mediated gene transfer of Apo2L/TRAIL in a model of antigen-induced arthritis in rabbits ameliorated the disease. In this study, apoptosis of synoviocytes rather than lymphocytes is described (89). On the other hand, Liu *et al.* described that collagen-induced arthritis in mice was ameliorated by injection of collagen-pulsed dendritic cells transfected with a doxycycline-inducible-Apo2L/TRAIL adenovirus system, reducing lymphocyte infiltration (87). More recent reports have shown that recombinant Apo2L/TRAIL directly ameliorates a rabbit knee model of arthritis (90) and is also able to suppress experimental autoimmune encephalomyelitis in mice (91).

Our group has performed over the last two years one study analyzing $CD3^+$ T cells present in the synovial fluids of 62 rheumatoid arthritis (RA) patients, using as controls synovial fluids obtained from patients with traumatic arthritis (TA). We confirmed that T lymphocytes present in the synovial fluids of RA patients have a chronically activated phenotype, but contrary to normal T cell blasts, they are resistant to Fas-induced toxicity. However, we showed that they are more susceptible to rApo2L/TRAIL than T cells in the synovial fluids of TA patients. In agreement with our data obtained with normal human T cell blasts (64), the effect of Apo2L/TRAIL was restricted to $CD8^+$ T cells. In addition, we found very low amounts of bioactive FasL and Apo2L/TRAIL associated with exosomes in the synovial fluids of RA patients as compared with those obtained from TA patients, which could account for the persistence of these T cells in spite of their sensitivity to Apo2L/TRAIL. Our data, obtained for the first time using cells from RA patients, indicate that bioactive Apo2L/TRAIL could be beneficial as a RA treatment also in humans, and especially in the late stages of the disease (92).

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