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COEVOLUTION OF HOSTS AND MICROORGANISMS: AN ANALYSIS OF THE INVOLVEMENT OF CYTOKINES IN HOST-PARASITE INTERACTIONS

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Parasites may employ particular strategies of eluding an immune response by taking advantage of those mechanisms that normally guarantee immunological self-tolerance. Much in the way as it occurs during the establishment of self-tolerance, live pathogens may induce clonal deletion, functional inactivation (anergy) and immunosuppression. At this latter level, it appears that certain pathogens produce immunosuppressive cytokine-like mediators or provoke the host to secrete cytokines that will compromise the anti-parasite immune response. It appears that immune responses that preferentially involve T helper 1 cells (secretors of interleukin-2-and interferon-γ) tend to be protective, whereas T helper 2 cells (secretors of IL-4, IL-5, IL-6, and IL-10), a population that antagonizes T helper cells, mediate disease susceptibility and are involved in immunopathological reactions. Cytokines produced by T helper 2 cells mediate many symptoms of infection, including eosinophilia, mastocytosis, hyperimmunoglobulinemia, and elevated IgE levels. Administration of IL-2 and IFN-γ has beneficial effects in many infections mediated by viruses, bacteria, and protozoa. The use of live vaccinia virus might be an avenue for the treatment of or the vaccination against infection. We have found that a vaccinia virus expressing the gene for human IL-2, though attenuated, precipitates autoimmune disease in immunodeficient, athymic mice. Thus, although T helper 1 cytokines may have desired immunostimulatory properties, they also may lead to unwarranted autoaggressive responses.

Key words: host-parasite interactions – tolerance – cytokines – interleukin 2

HOST-PARASITE INTERACTIONS LEADING TO IMMUNE TOLERANCE

The immune function is performed by a highly organized system that is endowed with certain characteristics traditionally thought to be the prerogatives of the central nervous system: recognition, (self-non-self) discrimination, learning and memory. The immune system is continuously confronted with the vital challenge to avoid aggressive reactions against self-structures, but to effectively eliminate the altered self (e.g. tumor cells) and to combat alien invadors (viruses, bacteria, protoozoa, etc.). A quasistochastic process (DNA rearrangement plus junctional diversity) generates the variability in the specificity of immunological recognition molecules by selecting different combinations of alternative protein cassettes in the immunoglobulin (Ig) heavy and light chains and α/β or γ/δ subunit genes of the T cell receptor (TCR). By consequence, antigen receptors of freshly generated, unselected lymphocytes have random specificities that, in principle, include both self and non-self antigens. Under normal circumstances, a variety of different mechanisms arranged in a fail-safe hierarchy guarantee that self-reactive, i.e. potentially auto aggressive cells will not be able to do harm to the self. Thus, on the first level, self-reactive T and B cells may be physically eliminated in their primary differentiation organs (thymus and bone marrow, respectively), a process that is generally referred to as clonal deletion. Clonal deletion that involves the activation of biochemical programs leading to the death (appoptosis) of the cell has also been described for peripheral T lymphocytes. On the second level, non-deleted self-reactive T and B cells will be activated under normal circumstances in a fashion that precludes their complete differentiation into effector cells, i.e. they are rendered "anergic". Finally, on the

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third level, non-deleted and non-anergic T cells will be subject to different immunosuppressive mechanisms that may involve cognate interactions ochestrated in terms of the idiotype-anti-idiotype network, or alternatively may concern antagonistic lymphocyte populations like the Th1 and Th2 subsets. The overall structure of this system that involves chronologically separate steps connected in series (deletion, anergy, and suppression) may explain some of the fundamental aspects of auto immune diseases, namely their polyetiology, their intermittent chronicity, and the relative easiness of therapeutical interventions (Kroemer & Martínez-A., 1991).

During the coevolution of parasites and hosts the former have developed strategies to prevent the generation of or to escape from efficient immune responses. On one hand, parasites may mimick host antigens (masquerade), vary continuously their antigenic characteritistics to elude immune recognition (camouflage) or use a network of cross-reacting epitopes on surface antigens to divert the immune system away from the production of high affinity antibody responses (diversion), as seen in the hypergammaglobulinaemia associated with chronic malaria (Anders & Smythe, 1989). On the other hand, different microorganisms, as well as multicellular parasites, may take advantage of the mechanisms that normally allow the establishment of immunological self-tolerance, mainly clonal deletion, functional inactivation or suppression of specific lymphocytes (Table). A key example for such a strategy is provided by certain retroviruses that have developed such an intimate relationship with their respective host that some of them incorporated themselves into the host's genome. Such retroviruses, as exemplified by the mammalian mammary tumor virus (MMTV) or the murine leukemia virus (MuLV) encode orf (open reading frame) proteins that lead to the intrathymic clonal deletion of relatively large T cell populations expressing certain TCR Vβ gene products (Acha-Orbea et al., 1991). Similarly, a number of pathogenic bacteria from the staphylococcal, streptococcal, as well as mycoplasma genera secrete so called enterotoxins (Herman et al., 1991) that lead to an initial stimulation of T

TABLE

Strategies of parasites to induce immune tolerance

Mechanism	Parasite (example)	Reference
Induction of clonal deletion or anergy via superantigens	Retroviruses Bacteria	(Acha-Orbea & Palmer, 1991) (Herman et al., 1991)
Induction of peripheral unresponsiveness	*Wuchereria bancrofti*	(Nutman et al., 1987)
Immunosuppression by secretion of an IL-10 analogue	Eppstein Barr virus	(Hsu et al., 1991)
Immunosuppression by secretion of a soluble TNF receptor analogue	Shope fibroma virus	(Smith et al., 1990)
Immunosuppression by excretory/ secretory products	*Brugia pahangi*	(Miller et al., 1991)
"trypanosoma immunosuppressive factor"	*Trypanosoma cruzi*	(Kierszenbaum et al., 1989)
inhibiting IL-2Rα expression	*Trypanosoma brucei rhodesiense*	(Kierszenbaum et al., 1991)
Immunosuppression by induction of host IL-10 production	*Schistosoma mansoni*	(Fidel & Boros, 1991)
Immunosuppression by induction of host TGF-β production	Human immunodeficiency virus	(Kekow et al., 1990)
Immunosuppression by induction of CD8+ suppressor T cells	*Echinococcus multilocularis* *Plasmodium falciparum*	(Kizaki et al., 1991) (Riley et al., 1989)
Elicitation of auto-destructive idiotype-anti-idiotype loops	Human immunodeficiency virus	(Martínez-A. et al., 1988) (Hoffmann et al., 1991)

cells that subsequently are clonally deleted (Kawabe & Ochi, 1991; MacDonald et al., 1991; Gonzalo et al., 1992). An example of intrathymic clonal inactivation is provided by the pre- or neonatal infection of mice with lymphocytic choriomengitis virus (Pircher et al., 1989) and Gross murine leukemia virus (Korostoff et al., 1990), although in these latter cases the timing of infection is important and adult stage exposure will not lead to tolerance. Also, parasites may induce peripheral unresponsiveness as seen in the parasite-specific B cell anergy of patients with asymptomatic microfilaremia (Nutman et al., 1987).

Microorganisms also may provoke general immunosuppression via idiotypic interaction. Human immunodeficieny virus (HIV) could subvert the host immune system by disturbing the idiotypic network. For example it has been hypothesised that the destruction of CD4+ T cells might be mediated by mirror-image anti-(anti-gp 120) antibodies (Martínez-A. et al., 1988). Another version of the same theory has been proposed that HIV, in combination with allogeneic stimuli, may induce a self-perpetuating destructive idiotype-anti-idiotype feedback loop that involves gp120, CD4, MHC class II products, anti-gp120 antibodies and the respective mirror-image anti-anti-gp120 idiotypes, thus leading ultimately to a functional paralysis and destruction of CD4+ cells (Hoffmann et al., 1991). It is conceivable that elimination of CD4+ T cells could occur by programmed cell death induced by inappropriate signalling resulting from the binding of anti-CD4 antibodies (Ameisen & Capron, 1991). Along the same line, the polyclonal B cell response to certain parasites has also been suggested to be a result of the disturbance of the idiotypic network, for example in the polyclonal antibody response to *Schistosoma mansoni* (Lopes et al., 1990). In addition, misdirection of network regulation to the parasite could explain the non-aggressive response to *Mycobacterium leprae* in lepromatous leprosy (reviewed in Cohen & Young 1991).

Pathogenic microorganisms may encode soluble immunosuppressive products. Thus, the gene encoding the Epstein-Barr virus protein BCRF1 (Baer et al., 1984) is homologous to IL-10 (Moore et al., 1990) and its expressed product, like IL-10, inhibits IFN-γ synthesis by antiviral lymphoid cells (Hsu et al., 1991). A transcriptionally active open reading frame from Shope fibroma virus encodes a product, the T2 protein, that shares 40% identical amino acid residues with the human TNF receptor (Smith et al., 1990). This product could be secreted and neutralize TNF α and β produced locally. Excretory/secretory products of the adult *Brugia pahangi* worm have been shown to cause the suppression of antigen-driven proliferation in previously infected lymph nodes of dogs (Miller et al., 1991). In addition, infectious agents may provoke the production of immunosuppressive factors by host cells. Thus, soluble egg antigen from *S. mansoni* induces the production of antigen-specific suppressive factors (possibly IL-10) by CD4+ T cells that inhibits the transcription of the IL-2 gene (Fidel & Boros, 1991). *Trypanosoma cruzi* induces the production of a factor, "trypanosoma immunosuppressive factor", that selectively inhibits the expression of the IL-2R α chain on human T cells (Kierszenbaum et al., 1991). This mechanisms is also used by *Trypanosoma brucei rhodesiense* to suppress the proliferation of human T lymphocytes in response to mitogenic stimulation (Kierszenbaum et al., 1991). Leukocytes from donors infected with HIV produce increased levels of the immunosuppressive cytokine transforming growth factor β (TGF-β) (Kekow et al., 1990).

In synthesis it appears that parasites developed a wide panel of different strategies to trick the immune system. One particular strategy takes advantage of the mechanisms normally guaranteeing self-tolerance, i.e. deletion, anergy, and immunosuppression.

CYTOKINES IMPLICATED IN HOST-PARASITE INTERACTIONS

Two kinds of soluble products are involved in the mediation of immune responses, namely antibodies and cytokines. Cytokines are implicated in the bilateral relations between the parasite and the host. They regulate the immune response, thus determining in part whether infection will result in immunological tolerance, immunity, or immunopathology. Cytokines cause many diagnostically relevant symptoms, e.g. IgE hyperemia (IL-4), hyperimmunoglobulinemia (IL-6), esoinophilia (IL-5), mastocytosis (IL-3, IL-4) fever (IL-1, TNF) and acute phase protein responses (IL-1, IL-6, TNF) (Finkelman et al., 1991; Stadnyk & Gauldie, 1991). The quantitation of cytokine serum levels may be of diagnostic and prognostic value and may facilitate the evaluation

of ongoing immune activation and inflammatory disorders. Increases in cytokine levels should precede the above mentioned biochemical or clinical disease symptoms, thus offering the possibility of expediting diagnostic procedures, as well as subsequent therapeutical interventions. Decreases in the concentration of cytokines – which are in general short-lived substances – would provide an objective criterium for assessing the therapeutic efficiency of medication.

The CD4+ peripheral T cell populations of the mouse may be divided in several subsets that differ in their lymphocyte secretion pattern. Thus, T helper 1 (Th1) cells, that are of the "inflammatory" phenotype, release interferon-γ (IFN-γ) and IL-2, whereas T helper 2 (Th2) cells ("helper" phenotype) release IL-4, IL-5, IL-6, and IL-10; further T helper subsets (Th precursor, Th0, ThX) exist (Mosmann & Coffman, 1989; Swain, 1991). Th1 and Th2 cells antagonize each other. Thus, IFN-γ and Il-2 produced by Th1 cells serve as functional antagonists of IL-4 (Bárcena et al., 1991). IFN-γ inhibits the expansion of Th2 cells. On the other hand, Th2 cells produce IL-10, which suppresses cytokine production by Th1 cells indirectly, i.e. via exerting effects on the antigen-presenting macrophage (Fiorentino et al., 1991). Filarial antigen stimulated IgE production in patients with filariasis is mediated by IL-4 and downregulated by IFN-γ (King et al., 1990). Similarly filaria-induced eosinophilia is associated with a significantly elevated frequency of IL-5-producing T cells (Limaye et al., 1990). Recent evidence suggests that CD4+ human T cells also may be separated into different Th types (del Prete et al. 1991; Parronchi et al. 1991). Human T cells specific for helminth (*Toxocara canis*) antigens are predominantly Th2 cells (Parronchi et al., 1991).

The immune response of a given host may become "locked" in either the Th1 or Th2 direction (Street et al., 1990). Several factors, including antigen concentration, administration route, MHC and non-MHC host genes determine whether a given antigen will induce preferentially a Th1 or Th2 response. As a general rule – that, of course, has its exceptions (Finkelman et al., 1991) – it may be stated that Th1 cells participate in protective immunity, whereas Th2 cells result in susceptibility to infection or mediate imunopathological manifestations (Grau & Modlin, 1991; Scott & Kaufmann, 1991).

Much information on the relative role of Th1 and Th2 cells in infection has been obtained in the mouse system. Two H-2 congenic strains, AKR and B10.BR exhibit disparate responses to the parasitic nematode *Trichinella spiralis*. AKR mice that expel intestinal worms produce more IFN-γ and specific IgG2a antibodies, whereas disease-susceptible B10.BR mice produce more IL-4, IgE, and specific IgG1 antibodies (Pond et al., 1989). Rejection of *T. spiralis* may also be related to the amount of IL-2 produced, as well as to the capacity of the lymphocytes of different mouse strains to respond to IL-2 (Kierszenbaum et al., 1989). In analogy, mouse strains resistant to *Leishmania major* infection, such as C57BL/6 and C3H/HeN exhibit delayed-type hypersensitivity to parasite antigen and expansion of IFN-γ-producing T cells in draining lymph nodes, whereas the susceptible BALB/c strain exhibits and increase in IL-4-producing T cells concomitant to the development of hyperglobulinemia and elevated IgE levels (Locksley & Scott, 1991). *Leishmania major*-specific lymphocytes isolated from infected BALB/c mice have been shown to produce elevated levels of IL-4 and decreased levels of IFN-γ in comparison to lymphocytes from resistant B10.D2 mice (Boom et al., 1990). Interestingly the administration of anti-IFN-γ antibodies to C3H/HeN mice during infection with *L. major* completely abolished their normal resistance to the parasite (Belosevic et al., 1989), whereas treatment of BALB/c mice with anti-IL-4 antibodies during similar infection attenuated and in most cases resolved the infection, with the establishment of protective immunity (Sadick et al., 1990). Immunization with peptides that preferentially induces Th1 (not Th2) clones may significantly enhance resistance of infection with *L. major* (Jardim et al., 1990; Yang et al., 1990), whilst immunization with a *L. major* repetitive peptide which activates Th2 cells enhances disease progression in BALB/c mice (Liew et al., 1990). In addition T cell lines (Scott et al., 1988) and clones of the Th1 phenotype (Scott et al., 1990) specific for protective *L. major* antigens are able to transfer protective immunity against infection to BALB/c mice, while lines specific for non-protective antigens exacerbate the disease (Scott et al., 1988). A role for antigen presentation in leishmaniasis has been suggested by the finding that the same antigen injected intravenously or subcutaneously induces protective and counter-protective T cells respectively (Liew, 1989). In a mouse model of helminthic infec-

tion with *S. mansoni*, Th1 cells clear the infection, whereas Th2 cells exacerbate the disease, supposedly by down regulation of the beneficial Th1 response via the secretion of IL-10 (Pearce et al., 1991).

CYTOKINES AS THERAPEUTICAL AGENTS IN INFECTION

Based on the previous considerations, it appears possible that cytokines produced by Th1 cells like IL-2 and IFN-γ might directly mediate the protective effect of this subset. The importance of IFN-γ, either alone or in association with other cytokines, for the generation of anti-viral immune responses and viral clearance has been documented for a number of viral infections including lymphocytic choriomenigits virus (Leist et al., 1989), vaccinia virus (Karupiah et al., 1990), Epstein-Barr virus (Hasler et al., 1983; Lotz et al., 1985), influenza A virus (Taylor et al. 1989), and *L. major* (Liew et al. 1989; Liew et al., 1990). The IFN-γ effect can be neutralized by IL-4 (Lehn et al., 1989), thus providing a speculative explanation of the disease-exacerbating effect of Th2 cells (Locksley & Scott, 1991). IL-2 synergistically enhances IFN-γ induction of reactive oxygen intermediates and killing of the protozoon parasite Giardia by human monocytes (Wahl et al., 1988). IL-2 moreover cooperates with IFN-γ in the induction of mouse macrophage resistance to infection with *L. major* (Belosevic et al., 1988) and cytostasis of *Leishmania mexicana amazonensis* in infected monocytes (Ho et al., 1990).

Based on the above studies, IFN-γ and or IL-2 were tested in clinical trials. Intralesional IFN-γ treatment of patients with leishmaniasis leads to a reduction in lesion size and parasitic load. This is observed for infections with either *Leishmania braziliensis guyanensis* and *Leishmania tropica* (Harms et al., 1989). A combination of IFN-γ and pentavalent antimony is effective in treating seriously ill patients with visceral leishmaniasis (Badaro et al., 1990). Intranodular injections of IL-2 also has beneficial effects on human disseminated cutaneous leishmaniasis, leading to a prominent influx of CD4+ T cells and clearance of amastigotes (Akuffo et al., 1990). Local administration of IL-2 reduces the systemic parasite lode in lepromatous lepra by increasing the influx of immunocytes from the circulation, the enhancement of cell-mediated immunity and the degradation of *Mycobacterium leprae* (Kaplan et al., 1989). Apparently, IL-2 can reduce the total body burden of leprosy bacilli when combined with multidrug chemotherapy (Kaplan et al., 1991). In mice, IL-2 may reverse impaired delayed-type hypersentivity to PPD induced by intraneous infection with *Mycobacterium tuberculosis* (Colizzi et al., 1988). IL-2 protects neonatal mice from lethal Herpes simplex infection in an IFN-γ-dependent fashion (Kohl et al., 1989). Moreover, IL-2 may protect mice from fatal *Escherichia coli* septicemia (Goronzy et al., 1989), infection by *Klebsiella pneumoniae* (Iizawa et al., 1988), or *Mycobacterium avium* (Bermudez et al., 1989). In contrast to the above data, Th1 products are not always beneficial to the host. Thus, anti-IFN-γ antibody treatment prevents the development of cerebral malaria in mice (Grau et al., 1989).

Other lymphokines than IL-2 and IFN-γ also are involved in protective responses. Acute non-A, non-B hepatitis may be prevented from becoming chronic by treatment with IFN-β (Omata et al., 1991). Granuloma formation in the liver of mice infected with *Bacillus Calmette Guerin* coincides with local TNF synthesis. Injection of anti-TNF antibodies 1-2 weeks after infection interferes with development of granulomas and subsequent mycobacterial elimination (Kindler et al., 1989). Similarly, TNF plays a protective role in experimental murine cutaneous leishmaniasis (Samuelson et al., 1991). TNF-α released by polymorphonuclear neutrophils stimulates the antifungal activity of these cells in an autocrine fashion (Djeu et al., 1986, 1988). In addition to mediating disease protection, TNF also may be implicated in pathological consequences of mycobacterial infection, namely caseation necrosis in tuberculosis, erythema nodosum leprosum, and nerve damage in leprosy lesions (Grau & Modlin, 1991).

AN INTERLEUKIN 2 VACCINA VIRUS CONSTRUCT AS A LIVE DRUG DELIVERY SYSTEM

Vaccinia virus (VV), the prototype member of the genus *Orthopoxvirus* is the first live organism that has been used for vaccination. Due to the virtual disappearance of small pox, VV is not used any more in its native state. However, the possibility to insert multiple foreign genes into VV and to use such recombinant virus in vaccination programs has led to a renaissance of VV biology. VV has a double-stranded DNA genome of approximately 185

kb in which large inserts of foreign DNA (up to 25 kb) may be introduced by homologous recombination (for review see Moss, 1991). Recombinant vaccinia virus (VV) constructs are used in immunology to express a wide panel of genes either *in vivo* or in cell culture systems to elicit humoral or cytotoxic responses (Murray et al., 1990) or to measure the effect of the expressed products on the host's immune parameters (Flexner et al., 1987; Gutierrez-Ramos et al., 1990; Andreu-Sánchez et al., 1991). VV recombinants have been designed to induce protective immunity against a variety of live pathogens in animals (Moss et al., 1984; Wiktor et al., 1984; Mackett et al., 1985; Yasuda et al., 1990); similar attempts are forthcoming in humans to induce protective immunity against the p160 glycoprotein of human immunodeficiency virus (Cooney et al. 1991). The lymphocyte tropism of VV has been addressed in a recent study. The VV's host range is extremely broad, comprising mammalian and avian species, and concerns many different cell types, because the infection does not require interaction with a specific surface receptor. However, upon *in vivo* infection, VV is not equally distributed throughout the organism, but is preferentially encountered in certain organs (Karupiah et al., 1990). Accordingly, B and T cells are not equally susceptible to VV infection, expression, and replication. β-galactosidase expression is less pronounced among T than B cells infected by a recombinant vaccinia virus (VV) that contains the gene of *E. coli* β galactosidase. VV infection caused a more pronounced growth inhibition of B cells and replicates better in B cells, as compared to T lymphocytes. Nonetheless, T cells do express proteins encoded by recombinant VV (Alonso et al., 1991). This suggests that Vv may be used to target the expression of genes to virtually every cell type. We have used IL-2. VV as a therapeutical agent capable of reducing *in vivo* the frequency of pro-autoimmune T cells in the MRL/Mp-*lpr/lpr* strain of mice. Repeated injection of IL-2. VV prevented the phenotypic manifestation of the *lpr* mutation that normaly entails a massive lymphadenopathy and an accumulation of CD3/TCRα/β+CD4-CD-8- T cells in the periphery. Concomitantly, IL-2.VV precluded the *lpr*-dependent development of autoimmune symptoms (Gutierrez-Ramos et al., 1990).

A major concern in the use of VV and VV recombinants for vaccination purposes are the adverse reaction rates and the fact that immunocompromised patients, including those with aquired immune deficiency may develop complications like disseminated vaccinia. Deletion of certain vaccinia virus genes, including thymidine kinase, hemagglutinin, or ribonucleotide reductase all decrease the virulence of vaccinia virus in experimental animals (reviewed by Moss, 1991). Alternatively, immunostimulatory cytokine genes may be introduced into the VV genome in their cDNA configuration, placing them under the control of the endogenous early/late p7.5 promoter that permits expression of the respective cytokine practically throughout the life cycle of the virus. This strategy has been employed for IL-2 (Flexner et al. 1987; Ramshaw et al., 1987), IL-1 (Ruby et al., 1991), IFN-γ (Kohonen-Corish et al., 1990), and TNF-α (Sambhi et al., 1991), resulting in attenuated viruses that are less pathogenic than wild type virus for immunodeficient athymic (*nu/nu*) mice. The injection of wild type vaccinia virus or a recombinant construct expressing *E. coli* β galactosidase, influenza hemaglutinin or nucleoprotein and/or *Herpes simplex* kinase into outbred Swiss *nu/nu* or inbred BALB/c *nu/nu* mice is lethal. In contrast, if human or murine IL-2 is expressed by the virus, mortality is significantly reduced; a finding which correlates with an accelerated clearance of the virus (Ramshaw et al., 1987), enhanced antibody titers (Flexner et al., 1987), elevated NK cell activities, IL-2 mediated induction of IFN-γ (Karupiah et al. 1990), and cytotoxic T lymphocyte responses (Mizuochi et al., 1989), and promotion of thymocyte differentiation (Kroemer et al., 1991a).

Given that IL-2 has a strong pro-autoimmune potential in man (Kroemer et al. 1986; Kroemer & Wick, 1989), we tested whether repeated administration of a VV recombinant containing the human IL-2 gene (IL-2.VV) might induce autoimmune side effects. Intraperitoneal injection of 1 x 10^7 plaque forming units of wild type vaccinia virus killed 10 to 12 week old athymic BALC/c *nu/nu* and C57Bl/6 *nu/nu* during the second week after treatment (Gutierrez-Ramos et al., 1992). In contrast, the same dose of recombinant human IL-2/vaccinia virus (vCF13, IL-2.VV) allowed survival of all animals, although repeated injection (2 week intervals) of IL-2.VV also limited the life span of athymic mice. Most animals succumbed to generalized dystrophia several days after the third injection of IL-2.VV. This premature death correlates with an

autoimmune phenotype (circulating rheumatoid factors, as well as anti-single-stranded DNA antibodies, proteinuria, and a severe Coombs-positive progressive erythroleukopenia). In contrast to athymic BALB/c *nu/nu* mice, euthymic controls did not develop autoantibodies, nor proteinuria, and survived several cycles of IL-2.VV treatment. Thus, in this model the induction of autoimmune phenomena by IL-2 is critically dependent on the absence of a thymus (Gutierrez-Ramos et al., 1992). Although even repeated administration of IL-2.VV was not lethal for neonatally thymectomized mice, also in this case autoimmune manifestations develop (autoantibodies, interstitial nephritis, proteinuria) (Andreu-Sánchez et al., 1991). Again, no adverse effects were found in euthymic controls. The probable explanation for the autoimmune disease-inducing effect of IL-2 in athymic conditions is that extrathymic T cell generation yields a largely non-deleted T cell repertoire, i.e. T cells that under normal conditions would be clonally deleted in the thymus due to their unwarranted self-specificity will accumulate in the periphery. Such cells are rendered anergic (functionally incompetent) via peripheral mechanisms, but IL-2 rescues these cells from the anergic state and enables them to lauch an autoaggessive attack (Kroemer et al. 1991 a, b; Kroemer & Martínez-A., 1991). Accordingly, high frequencies of non-deleted "forbidden" T cells expressing certain TCR Vβ gene products that normally are deleted due to their reactivity with self-superantigens encoded by endogenous retroviruses are encountered in constitutively athymic or neontally thymectomized mice. When recovered from untreated mice or controls receiving a wild type VV, such cells are incapable to proliferating or delivering help to B cells upon crosslinking of the "forbidden" TCR. Nonetheless, after in vivo IL-2.VV treatment, these cells readily proliferate and enhance immunoglobulin production by B cells (Andreu-Sánchez et al., 1991; Gutierrez-Ramos et al., 1992). It is conceivable that imbalances in distinct T cell populations, altered helper/suppressor ratios, a deficient lymphokine-absorptive capacity ("lymphokine sink") due to lymphopenia, or the inexistence of an elaborated idiotypic network may contribute to the autoimmunity predisposing effect of athymia. In view of the pro-autoimmune effect of IL-2.VV, we conclude that this recombinant would be contraindicated in immunodeficient individuals, as well as in persons with latent autoimmune diseases. This illustrates the need to characterize the immune state of a given individual prior to administration of a cytokine.

In contrast, IL-2.VV appears relatively safe in immunocompetent individuals, and this self-replicating live drug delivery system may be taken advantage of in two strategies. First, IL-2.VV incorporating antigen-encoding genes may provoke efficient immune responses due to the "adjuvant effect" of IL-2 that allows the convertion of non-responders into responders. Thus, low-dose-IL-2 has been shown to allow for the elicitation of antibodies in hepatitis-vaccinated non-responders (Meuer et al., 1989). In addition, systemic IL-2 levels provoked by IL-2.VV (Gutierrez-Ramos et al., 1990) could stimulate the immune system much in the way as constant IL-2 infusions, thus promoting T cell differentiation and the generation of a new T cell repertoire. In view of the capacity of IL-2 to enhance anti-parasitary reactions (*vide supra*), this IL-2.VV effect may warrant further investigation. Whether delivered by life organisms or in a more conventional form, cytokines might be for the twenty-first century what antibiotics have been for the twentieth.

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