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Suppressor mechanisms in tumor immunity

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Summary. There are many parallels between T cell-mediated suppression of tumor immunity and suppression of immune responses to haptens and polypeptides. We propose a cell interaction model which takes this into account and outlines a regulatory pathway for suppression of immunity to tumor antigens. Free antigen or antigen/antibody complexes trigger an inducer T cell subset, Ts^i, which is tumor-specific. This cell activates a non-immune T cell population, pre Ts^e, to generate effector suppressor cells, Ts^e. The Ts^e are specific for either the idiotype of Ts^i or for antigen complexed with a soluble factor made by the Ts^i, but the suppression they mediate is antigenically nonspecific. Tumor antigen-specific suppressor factors, TsF, play a major role in the communication between different suppressor cells. Characterization of polyclonal and monoclonal factors produced by Ts^i, called TsF^i, indicates that they both bind to tumor antigen and contain tumor-specific (idiotypic?) determinants.

Introduction

The demonstration, almost 15 years ago, that tumor immunity can be specifically suppressed by sera of tumor-bearing mice provided one of the first examples of immunoregulation by soluble factors (Hellström et al.[37]). Numerous explanations were proposed to account for this suppression. They included facilitation of tumor growth by 'enhancing antibodies' (Möller[55], Voisin[91], Kaliss[50]), as well as inhibition of tumor immunity by soluble tumor antigen (Alexander[1], Brawn[11], Vaage[90], Baldwin et al.[5]), antigen-antibody complexes (Sjögren et al.[77], Baldwin et al.[4], Gershon et al.[25]), and T cell-derived factors (Nelson et al[61], Umiel and Trainin[89], Greene et al.[31]). As work progressed, several of the original hypotheses had to be modified, and in 1977 we introduced the concept of specific blocking factors (SBF) in an attempt to unify the available data (Hellström et al[42]). SBF were defined as antigen-specific factors present in sera of tumor-bearing hosts, in soluble extracts of tumors, or in supernatants from cultured tumor-derived T cells which suppressed the cytostatic potential of immune T cells in vitro and/or enhanced tumor growth in vivo. Although the precise relationship between tumor antigens (or complexes) and T cell-derived immunosuppressive factors was unclear, we postulated that the former acted as the proximate signal that triggered the latter, which then provided the effector signal needed to shut off the immune

Abbreviations used:
Ts, suppressor T cell
TsF, suppressor factor from a Ts
SBF, specific blocking factor
TAA, tumor-associated antigen
Cx, cyclophosphamide
MHC, major histocompatibility complex

response (Hellström et al.[42], Nepom et al[67], Nepom[65]). During the past 5 years, it has become apparent that tumor-specific suppression by T cells and by T cell-derived factors plays an important role in an antigenic tumor's escape from immunologic control, analogous in may ways to T cell-mediated suppression in general, as studied in models of immunity to haptens and polypeptides (Tada et al.[82], Pierce and Kapp[73], Germain and Benacerraf[22], Gershon[24]). It is now possible to construct a more comprehensive model for suppression of tumor immunity, in which soluble tumor antigen, antigen-antibody complexes, anti-idiotypic antibodies and T cell-derived suppressor factors, interrelate in a common pathway of immune regulation. In this review we outline this pathway, and discuss some of the major contributions which support it. Other recent reviews (Greene[28], Naor[59], Hellström et al.[45]) provide additional background for portions of our hypothesis. We will not discuss here aspects of antigen non-specific immunosuppression which also occur in association with tumor growth.

General outline of the model

Numerous parallels exist between suppression of tumor immuniy and the suppressor cell (Ts) network as described for immune responses to well-defined antigens, such as haptens and polypeptides. This implies that an antigenic tumor can 'use', in its escape from immunologic control, normal mechanisms of immune regulation, and that these may be aberrantly activated or inappropriately regulated. By analyzing suppression of tumor immunity on the basis of what is known about suppression in general, it should be possible to identify several critical steps in the regulatory circuit that can be manipulated towards development of therapy. We shall first outline the general principles of a model for suppression of tumor immunity and then discuss its individual parts in some detail.

Three different types of Ts can be identified which are involved in suppression of tumor immunity (table): An initial T cell, called inducer Ts, Ts^i, is the proximate recipient of a signal which emanates from the tumor; this signal is specific and is either the tumor antigen itself or a soluble immune complex containing the antigen. The role of the Ts^i is to recruit and/or amplify an effector suppressor population, called Ts^e, from non-committed T cells. The latter cells are here called pre-Ts^e, but they are sometimes also referred to as acceptor Ts, Ts^a (Cantor and Gershon[13]). Communication between Ts^i and pre-Ts^e is crucial for suppression to occur. Soluble factors, TsF, which can mediate this communication have been identified and are generated by the Ts^i. These factors, to the extent studied, are antigen specific and antigen binding, and they may carry MHC-encoded determinants. After the pre-Ts^e has received a recruitment signal in the form of the TsF^i, it evolves functionally into an effector suppressor cell, Ts^e. That cell appears to be the final cell which directly inhibits the function of immune helper and pre-killer T cells and/or other immune cell types. Since pre-Ts^e and Ts^e share several surface markers, it is not clear whether two different cellular subsets are involved, with a signal going from pre-Ts^e to Ts^e, or whether the pre-Ts^e differentiates into Ts^e.

We shall now discuss the various components of the model in some detail.

Induction signal

Antigen released by the tumor is likely to be the primary signal which is needed for activation of the suppressor pathway (Alexander[1], Vaage[90], Currie and Basham[16], Coggin[15]), and some oncofetal antigens (Castro et al[14], Hellström and Hellström[39]) appear to be particularly prone to shut off an effective response to themselves. Experimentally, suppression can be induced by antigen given in the form of soluble tumor extracts, heavily irradiated tumor cells, or immune complexes prepared by combining tumor extracts with antitumor antibodies (Baldwin et al.[4], Gershon et al.[25], Hellström and Hellström[36], Gorcznyski and Knight[27]).

The presence of circulating tumor antigen or antigen complexed with antibody has been documented in animals and human patients with tumors (Theofilopoulos et al.[86], Hofken et al[46], Brandeis et al.[10], Amlot et al.[2], Oldstone[68], Jennette and Feldman[47], Heimer and Klein[35]). Suggestive evidence that both complexes and free antigen can induce suppression was first obtained by Sjögren et al., who fractionated sera from tumor-bearing mice into small ($M_R < 100{,}000$) and large ($M_R > 100{,}000$ components) and observed that the fractions would suppress ('block') tumor immune responses in vitro when they were combined, or when the smaller fraction was tested alone. The larger fraction alone did not suppress. Since antibodies were present in the larger fraction, the data suggested that both antigen and soluble complexes could suppress cell-mediated anti-tumor immunity while free antibody could not (Sjögren et al.[77]).

Although tumor antigen, alone or as part of an immune complex, is sufficient to trigger the generation of Ts^i (Perry and Greene[70], Paranjpe et al.[69]), 2 experimental manipulations have been described which facilitate the ability of tumor antigen to suppress, namely exposure of animals to UV irradiation and treatment with anti-Ia serum. Both of these manipulations may work via a similar mechanism. Kripke's group demonstrated that sarcomas arising in UV-irradiated mice could be transferred to other mice only when these mice were UV-irradiated (Kripke[53]). This was found to be due to the fact that the UV-sar-

comas are highly antigenic and hence rejected by untreated mice but that prior UV-irradiation of the recipient induces Ts which, after tumor transplantation, prevent rejection (Fisher and Kripke[18]). One possible mechanism for this phenomenon was suggested by the finding that UV-irradiation selectively decreases the number of I-A positive spleen cells which can present antigen to carrier-specific T cells (Letvin et al[54]). Thus UV-irradiation may change the presentation of antigen so that it bypasses the normal Ia-dependent pathways to preferentially trigger the activation of Ts^i. Consistent with this interpretation is the demonstration that injection of an alloantiserum to I-A-coded products immediately before tumor transplantation could enhance tumor outgrowth (Perry et al.[71]). The serum effect was haplotype and subregion specific and probably due to inhibition of normal antigen-presenting pathways. Analogous examples can be found in non-tumor systems (Drebin et al.[17]), both in vivo (Sprent[78], Bromberg et al.[12], Rosenbaum et al.[76]) and in vitro (Berzofsky and Richman[9]). In view of these findings one would expect that the route of tumor presentation to the immune system will influence the extent to which suppression is induced, since intravenous injection of antigen may bypass the macrophages and preferentially induce suppression (Greene and Bach[29]).

Characteristics of Ts^i and TsF^i

Following induction by antigen (or antigen-antibody complexes) tumor-specific Ts^i activate pre-Ts^e into generating (or becoming) Ts^e. The Ts^i make TsF^i which play a crucial role as activation signals to the pre-Ts^e.

There are several characteristic immunologic and biologic markers which identify Ts^i. One of their most important characteristics is that they are antigen-specific (Fujimoto et al.[20], Takei et al.[83], Asherson and Zembala[3]) and possess specific antigen receptors at their surface (Greene[28]). The presence of receptors for antigen appears as a natural consequence of the Ts^i being induced by antigen and may distinguish them from Ts^e.

Tumor-specific soluble factors, TsF, have been described which selectively bind to unique tumor antigens, so that their suppressive activity can be removed by absorption onto appropriate tumor cells in vitro. This was demonstrated initially with affinity-purified TsF specific for two different chemically induced fibrosarcomas in mice, where the TsF was obtained from sera of tumor-bearing animals (Nepom et al.[66,67] and subsequently with a monoclonal TsF obtained from a T cell hybridoma produced by fusing thymocytes from a tumorbearing animal with BW5147, an azaguanine-resistant thymoma (Nelson et al.[63]). The latter factor can suppress tumor destruction by immune T cells in vitro, enhance tumor outgrowth in immune syngeneic mice in vivo and inhibit delayed-type hypersensitivity reactions to the tumor (Hellström et al.[44], Nelson et al.[64]). Other antigen-specific TsF have been decribed, prepared from lysates of thymocytes (Greene et al.[31]) or from supernatants of cultured splenic T cells from mice with growing tumors (Nelson et al.[60,61]) which are similar (identical?) to these antigen-binding factors. It seems likely that the activity of Ts^i is generated by way of such antigen-specific soluble factors, analogous to the potent immunosuppressive activity characteristic of TsF^i which bind antigen and amplify suppression of immunity to model antigens, such as the haptens ABA, TNP and NP and polypeptides GAT and KLH (Tada et al.[82], Pierce and Kapp[73], Germain and Benacerraf[22], Greene et al.[34], Moorhead[56]).

A 2nd characteristic feature of Ts^i and TsF^i is that both appear to bear determinants that are coded within the I-J subregion of H-2. Greene et al.[33] and Perry et al.[72] demonstrated that antisera to I-J products inhibited tumor outgrowth when administered intravenously to mice of the appropriate genotype; this was consistent with the hypothesis that a population of I-J+ suppressor cells was functionally deleted by the antiserum. Further evidence for an I-J determinant on TsF^i was obtained by Koppi et al.[52], who reported that soluble TsF^i were removed by immunoadsorption on affinity columns containing antibodies directed to I-J coded determinants.

A 3rd characteristic of Ts^i is that they are resistant to low dose irradiation and to cyclophosphamide, a finding which provides a practically useful way to distinguish them from pre-Ts^e. The evidence for this comes from several sources. Studying the mechanisms by which tumor growth can be enhanced by giving tumor antigen, Hellström et al performed cell transfer experiments which showed that tumor growth was facilitated by a population of antigen-specific splenic Ts that are resistant to 400 R (Hellström et al.[38].). Rao et al.[75] injected mice with complexes of antibodies and antigen from L1210 mouse lymphoma cells, and so generated Ts which could adoptively suppress tumor immunity. These cells, which were present in the spleen and expressed Lyt-1 antigen, were relatively resistant to radiation and cyclophosphamide. Precursors of this mature Ts^i, however, were found to be cyclophosphamide-sensitive. Mule et al.[57] detected Qa-1-positive, cyclophosphamide-resistant T cells in the spleens of tumor-bearing mice which functioned as 'suppressor activators' when transferred to mice challenged with tumor cells and were comparable to the Ts^i. In sum, the picture emerges of an Lyt 1+23-, I-J+, Qal+ splenic Ts which, along with its soluble product, TsF^i, adoptively transfers suppression in a tumor-specific fashion.

Amplification signal

Adoptive transfer experiments indicate that the Ts^i must recruit or amplify a different population of T cells, which we call pre-Ts^e, for generation of a mature suppressor effector cell, Ts^e. The signal that goes from Ts^i to the pre-Ts^e is antigen-specific. The molecule which mediates this signal, TsF^i, may be identical to one of the antigen binding factors previously identified in sera from tumor-bearing animals (Nepom et al.[66]), in supernatants from T cells cultured from the spleens of tumor-bearing mice (Nelson et al.[61]), and in supernatatnts from a T-T hybridoma (Nelson et al.[63]) obtained by hybridizing thymus cells from tumor-bearing mice (see above). Unresolved, however, is whether the signal is TsF^i alone or whether it is a combination of TsF^i with tumor antigen. In the former case, a receptor on pre-Ts^e cells would recognize an idiotypic determinant on TsF^i, while in the latter case it would recognize the antigen itself.

Although anti-idiotypic suppressor T cells have not yet been identified in tumor systems, there is ample precedent for such cells in hapten models (Greene et al.[34]). Furthermore, Nepom et al.[67] and Koppi et al.[52] have reported that sera from tumor immune mice contain antibodies with unique specificity for antigen-specific suppressor (blocking') factors. These observations suggest that TsF^i may possess idiotypic determinants which, when mice are repeatedly stimulated with tumor antigen, induce the formation of idiotype-specific antibodies. Whether the presumed appearance of anti-idiotypic antibodies is paralleled by the generation of anti-idiotypic T cells is not yet apparent. Experimental verification of this hypothesis would be a significant advance relating concepts of regulatory networks (Jerne[48]) to understanding tumor immunity.

An alternative hypothesis to the proposal that TsF^i acts as an idiotypic factor is that TsF^i associates with tumor antigen by virtue of its antigen specificity, and functions by presenting antigen to the pre-Ts^e; in this case, the pre-Ts^e would have anti-antigen specificity. There is precedent also for the latter model, in that suppressor circuits specific for the polypeptides GAT and GT appear to be mediated by a TsF^i which induces Ts^e only if complexed with antigen (Kapp and Araneo[51], Germain et al.[23]). In that case, as for TsF suppressing immunity to KLH (Tada and Okumua[81]), an antigenic stimulus is required for the generation of Ts^e, while such a stimulus is not needed in hapten systems, where idiotypic TsF^i is a sufficient stimulus (Sy et al.[80]).

Characteristics of pre-Ts^e and Ts^e

Many investigators have described a population of T cells that is both radiosensitive and cyclophosphamide sensitive and that is needed for suppression to occur. It can be detected in mice transplanted with tumor cells, alone or together with Ts^i (Hellström et al.[38,73], Rao et al.[75], Tilkin et al[87], Glaser and Law[26], Green et al.[32]), and it is present also in thymus and spleen from non-immune donors (Hellström et al.[38]). Rao et al.[75] have reported that the cell acting as recipient of the Ts^i signal is Lyt-1+23+.

The effector cell mediating suppression, Ts^e, is different from the pre-Ts^e in that it appears to be Lyt-1-23+ (Mule et al.[59]). It is not clear whether the Ts^e is a differentiation product from pre-Ts^e or whether it belongs to a separate subset (Cantor and Gershon[13]).

Although the Ts^e is triggered by an antigen-specific signal, its suppressive activity is non-specific. This has been demonstrated both in vivo and in vitro. In vivo, Hellström et al.[38] found that the rejection of a chemically induced sarcoma which expresses a unique tumor-specific transplantation antigen was suppressed not only by Ts^e specific for the same sarcoma but also by Ts^e specific for antigens of an unrelated sarcoma, if

Suppressor cell subsets in the tumor immune interaction model

Cell designation	Synonyms	Induction signal	Specificity	Ia marker	Cx	Irradiation	Lyt	TsF
Ts^i	Ts_1	Antigen (tumor or extract)	Tumor antigen	I-J+	Resistant	Resistant	1+	TsF^i
	Ts afferent							
	Ts activator	Antigen/antibody complex						
	Ts inducer							
Pre-Ts^e	Ts_2							
	Ts acceptor			?	Sensitive	Sensitive	1+23+	?
Ts^e	Ts_3	$TsF^i \pm$ antigen	Acts non-specifically	?	?	?	1−2+	?
	Ts effector	Activated specifically ?idiotype/?antigen						

these were activated by their appropriate Ts^i signals. In a similar system, Mule et al.[57] found that the development of mature Ts^e required antigen-specific triggering, while the resulting suppression was no longer restricted to a unique tumor specific transplantation antigen. Analogous observations have been made in vitro, since Bean et al. found that lymphocytes from tumor-bearing patients can be triggered to suppress responses to third-party antigens by exposure to cells from the patient's own tumor as an antigen-specific induction signal (Bean et al.[8]). In non-tumor systems, there is precedent for antigen-specific triggering followed by antigen-nonspecific suppression, as shown for allogeneic responses (Truit et al.[88]), as well as for carrier-specific (Taniguchi and Tokuhisa[84]) and hapten-specific (Sy et al.[79]) responses.

One unresolved question concerning Ts specificity is the nature of the antigenic determinant on the tumor cell which acts as an activation signal. Is this determinant the same as tumor-associated antigens (TAA) which act as targets for cytotoxic T cells or is it a separate 'suppressor' determinant? In most cases, TAA defined by rejection of syngeneic tumor grafts are individually unique among chemically induced sarcomas in mice (Prehn and Main[74]). The specificity of suppression mediated by purified soluble TsF^i has also been shown to be tumor specific and correlates with the specific TAA (Nepom et al.[67]). However, even in cases where the targets of cytotoxic lymphocytes are not unique for each tumor, the suppressor phenomenon seems to be more specific and able to discriminate between apparently cross-reactive tumors both in vivo, (Greene and Perry[30]) and in vitro (Fujimoto et al.[21]). This implies that the Ts recognition site may differ from other immune T cell antigen receptors and therefore that the TAA recognition by these two T cell subsets may differ.

Predictions of the model

One of the rationales for constructing a model for T cell suppression of tumor immunity is to use the model to suggest ways to manipulate the immune response in favor of the tumor-bearing host. As discussed, some experimental manipulations, including irradiation with UV light and injection of anti-Ia antisera, facilitate the induction of suppression. Other manipulations, such as whole body X-irradiation or treatment with cyclophosphamide, inhibit subsequent amplification steps generating suppression and can, under certain circumstances, inhibit tumor growth and even induce regressions (Hellström et al.[43]),

On the basis of the evidence that circulating antigen (and complexes) induce suppression, immunotherapy has been designed which is geared toward removing the circulating induction signal. In most cases, this therapy has been based on the ability of protein A adsorbants to remove antigen that is complexed with antibody. Such therapy has been effective in certain clinical situations (Bansal et al.[7], Terman et al.[85]), most strikingly in cats with FeLV-induced leukemias (Jones et al.[49]). Although there still are questions regarding its efficacy, a possible explanation is that protein A adsorption leads to the removal of antigen, that is, of the induction signal for suppression. Alternative interpretations include the removal of TsF as well as direct effects of protein A on the host (Hellström and Hellström[41]).

Since Ts^i can recruit (or amplify) a suppressor cell population from nonimmune pre-Ts^e cells, a possible target for experimental manipulation is to abrogate this recruitment (amplification). It seems likely that the early efforts at immunotherapy using 'unblocking sera', i.e., sera which abrogated the suppressive (blocking) activity of sera from tumor-bearing animals (Bansal and Sjögren[6]), when effective, interfered with this step, since 'unblocking' sera contain antibodies to circulating suppressor ('blocking') factors (Nepom et al.[67], Koppi[52]). Promising strategies to extend this type of experimentation will include the development of monoclonal antibodies to TsF determinants for potential therapeutic intervention. The use of anti-I-J antisera probably also interferes with this amplification step (Greene et al.[33], Perry et al.[72], Koppi et al.[52]).

One prediction of the model is that communication between interacting cells may be genetically restricted so that, for example, the signal from Ts^i to Ts^e is restricted across histocompatibility or allotype barriers. We are presently looking for such restriction, since it could identify communication events subject to other types of manipulation.

Another experimental approach suggested by the model is based on the possibility that some antigen-specific signals among Ts involve idiotype-antiidiotype interactions. If an idiotypic network does regulate Ts amplification, then manipulation by methods such as priming with anti-idiotypic antibodies may be useful. Tilken et al.[87] have reported use of an antisera to enhance tumor immunity which was raised against putative T cell anti-tumor receptors found on blastic T cells, although Flood et al.[19] found that a similar putative anti-idiotypic antiserum suppressed tumor rejection. Clearly, further definition of precise idiotypic and anti-idiotypic markers on T subsets is needed to design appropriate strategies for intervention. Further modifications of the model which we have outlined await the development of an intact network of cloned cells allowing more precise investigation. It should be possible to develop such a network with the advent of cell cloning techniques as exemplified by the recently reported T cell hybridoma which forms a tumor-specific TsF, as discussed above.

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The locus coeruleus: actions of psychoactive drugs

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Summary. The locus coeruleus is one of the most thoroughly investigated mammalian brain areas. Its fibers innervate large parts of the neuraxis, in particular, areas involved in cognitive functions such as the cortex and the hippocampus. A role of locus coeruleus has been proposed in such processes as memory, the control of vigilance, blood pressure and others. Results obtained in this and other laboratories demonstrate that the firing rate of locus coeruleus neurons is affected by a great number of psychoactive agents such as antidepressants, minor tranquillizers, neuroleptics, psychostimulants and certain psychogeriatric drugs. We have attempted to correlate the data obtained on the cell bodies of locus coeruleus with studies reporting effects on terminal areas and thereby gain an overall view of the action of the above mentioned drugs on this cell system. The activity of noradrenergic neurons in locus coeruleus is thought to correlate with the level of cortical vigilance. Special emphasis is placed on the finding that a number of drugs which exert a positive effect on cognitive functions in man and animals increase the firing rate of the rat locus coeruleus neurons.

Introduction

Since the discovery of noradrenaline in the locus coeruleus (LC) this group of neurons has received ever increasing attention by neurobiologists. Today the noradrenaline-containing LC-system is undoubtedly one of the best documented neuronal systems in the brain. The number of functions which have been proposed and the knowledge of the biological properties of this system which has been acquired in the past 5-10 years have grown enormously.

The proposed functions of LC encompass such diverse processes as memory and learning[2], attention[38,40,41], the sleep-wakefulness cycle[33], extinction[39] and others. In the past 2 years research has focussed again on the theory which states that this cell group is involved in the regulation of vigilance and attention[25,32,33,36,41]. Support for this notion has come from several lines of research. In single cell recording studies performed in freely-moving rats and monkeys it has been shown that the activity of LC-neurons correlates with the level of vigilance[4,12,22,30]. Highest levels of cellular activity were seen during wakefulness and lower firing rates were observed when the animals were drowsy or in slow-wave sleep. LC-neurons were activated by a variety of sensory stimuli[5] and changes in electrocortical activity were anticipated by changes in LC-neuronal firing activity[4,5]. The relationship between LC-neuronal activity and vigilance points to a link between arousal and the release of noradrenaline. Behavioral and electrophysiological evidence supports the argument that LC plays a role in attention[41]. In electrophysiological studies it has recently been shown that noradrenaline may facilitate the transfer of afferent information within the cerebral and cerebellar cortical circuitry by enhancement of the 'signal-to-noise' ratio[57,58]. Thus the LC-system may serve as a kind of 'filter' which separates behaviorally relevant from irrelevant, distracting signals and in this way augments and focusses attention. Further evidence for a role of the central noradrenergic systems in the control of arousal comes indirectly from pharmacological studies. The 'classical' catecholamine hypothesis of depression is based on the assumption that noradrenergic neurotransmission is linked with mood and behavioral alertness. The known noradrenaline potentiating property of classical antidepressants and of amphetamine is in keeping with this notion. Interestingly, classical antidepressants[42,48] and amphetamine[27] depress the spontaneous firing of noradrener-