Immunity to self and self-maintenance: what can tumor immunology teach us about ALS and Alzheimer’s disease?

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Mounting evidence from the last decade has shown that the immune system not only fights pathogens but also protects the body against cancer. More recently, immune surveillance has been shown to be important for maintaining the functional integrity of the central nervous system. The immune system, however, does not always prevail; tumors do grow and eventually kill their host, and devastating neurodegenerative conditions do develop. Neurodegenerative diseases, like tumors, lie dormant long before clinical symptoms appear. We propose that at this dormant stage, an ongoing competition between the local disease-causing factors and the immune system’s attempts to contain them takes place. Onset of clinical symptoms occurs after disease-causing factors escape immune surveillance. Identifying the immune escape mechanisms and circumventing them soon after the emergence of clinical symptoms could lead to the development of novel therapeutic intervention for some of the most devastating neurodegenerative disorders.

Working hypothesis
The concept of immune surveillance traditionally has referred to the body’s defense against pathogens. This concept has been extended to include defense against tumors, suggesting that the immune system is constantly on the lookout for aberrant cells and tumors and eliminates them before they can cause disease.

Studies by our group have introduced a novel non-classical role for the immune system in the context of the central nervous system (CNS): the maintenance of functional CNS integrity [1,2]. T cells recognizing self-antigens that act in concert with local innate immunity mediate this role of the immune system. The overall function of the immune system in repair, removal of aberrantly growing cells and regulation of cell renewal prompted us to propose a surveillance role of the immune system within the CNS (extensively reviewed in Ref. [3]). We suggest that escape from immune surveillance could explain emergence of CNS dysfunction in analogy to cancer emergence. According to our proposal, neurodegenerative diseases, in a manner similar to cancer, might be dormant long before the onset of symptoms. This proposal is based, in part, on the role of protective autoimmunity in CNS maintenance and repair [1,3,4].

Thus far, the immune system has been discussed in the context of CNS diseases only as a factor contributing to the pathology but never in the context of providing surveillance before disease onset. Our group has obtained a large body of evidence suggesting that under normal conditions, CNS immune surveillance, primarily mediated by T lymphocytes recognizing CNS antigens, is important for maintaining cognitive abilities and the capacity for neuronal cell renewal in adulthood [1,2,5–7]. The possibility that CNS pathologies emerge after a long stage of struggle between the disease pathology and the attempts of the immune system to fight it off (as in the case of cancer) has not been previously suggested. To gain insight into the possible stages at which such failure could take place, we examined whether the principles that guide immune surveillance in the context of tumors also are applicable to neurodegenerative diseases. We focus our discussion on amyotrophic lateral sclerosis (ALS) and Alzheimer’s disease.

How do tumors escape immune surveillance?
Tumor immune surveillance recently has been accepted as a key process by which the body prevents cancer [8,9]. There is now evidence suggesting that this phenomenon is part of a multistep process termed ‘tumor immunoediting’. The concept of tumor immunoediting, formulated by Dunn and Schreiber, comprises three consecutive phases: elimination, equilibrium and escape (‘the three Es’; for extensive reviews, see Refs [8,9]).

In the first step the immune system specifically detects aberrant cell growth and eliminates the tumorogenic cells. The elimination step is initiated by the activity of the innate immune response, which detects a tumor-associated danger signal [10,11]. Such a danger signal can take the form of proinflammatory cytokines derived from the tumor itself or from adjacent cells during tissue remodeling by the tumor [9]. The activity of the innate immune cells results in increased levels of interferon gamma (IFN-γ) that acts to restrict tumor growth by inhibiting proliferation and angiogenesis and by promoting apoptosis. IFN-γ also contributes to the recruitment of additional innate immune cells, which express tumoricidal products. These events, in turn, lead to the activation of dendritic cells (DCs, professional antigen-presenting cells), which present tumor antigens, thereby promoting a tumor-specific adaptive immune response. Tumor-specific T cells (CD4 and CD8)
migrate to the tumor site and contribute to specific ablation of cells that express tumor antigens. Certain products of apoptosis also can be perceived by the immune system as danger signals and contribute to additional immune activation. For example, uric acid, an end product of purine catabolism that exists in the extracellular environment as monosodium urate, is elevated in apoptotic tumor cells and was shown to elicit immune-mediated tumor rejection by activating DCs and monocytes [12–14].

If the elimination of tumor cells is incomplete, the antitumor immune response persists during a relative long period termed ‘equilibrium’. During this phase the immune response induces strong selective pressure on the tumor. This selective force restricts the growth of tumor cells that can be recognized by immune cells and, thus, enables the survival of only those clones of tumor cells that mutate so that they can no longer efficiently be recognized by immune cells [9].

This process slowly develops and brings about the final phase of the immune-mediated anticancer response, ‘escape’. In the escape phase tumor cells can grow without significant involvement by the immune system. It is usually during this phase that the tumor becomes clinically detectable [9].

Tumor-escape mechanisms can be grouped into two main categories: (i) factors intrinsic to the tumor (associated with the tumor cells and tumor-associated antigens) and (ii) extrinsic factors, affecting the host immune response and not directly related to the tumor [9]. Intrinsinc factors include: (i) the lack of expression of class II MHC proteins (MHC-II) and co-stimulatory molecules, (ii) downregulation or loss of expression of MHC-I proteins [15,16], (iii) downregulation or loss of expression of genes associated with antigen presentation [17], (iv) weak expression of tumor-associated antigens at early phases of tumor growth [18], (v) loss of antigenic epitopes, (vi) a physical barrier preventing access to tumors by effector cells, (vii) loss of response to IFNs [19–22] and (viii) upregulation of Toll-like receptor (TLR) expression on tumor cells [23,24].

The second group, involving factors associated with the host immune system, can include: (i) immune ignorance, (ii) T-cell suppression induced by tumor-derived factors [e.g. transforming growth factor (TGF)-β, IL-10, vascular endothelial growth factor, FasL, galectin] [25], (iii) secretion by the tumor of soluble ligands that block lymphocyte activation (e.g. NKG2D-L) [26], (iv) defects in antigen presentation by antigen-presenting cells (APCs), (v) impaired APC maturation [27,28] and (vi) tolerance of T cells to tumor-specific antigens or suppression of T-cell response by regulatory T cells [29–35].

Is immune surveillance for CNS protection similar to anti-tumor immune surveillance?

Little is known about the dialogue between the immune system and the diseased CNS at the preonset stage, namely prior to the emergence of the clinical symptoms.

Elimination

Until the last decade it was generally believed that any acute or chronic disorders of the CNS must be repaired by the CNS tissue alone and that any immune-cell activity at the site of damage would be insignificant at best or harmful at worst. We suggest that, as in the elimination phase of tumor immunediting, any deviation from homeostasis in the CNS triggers a cascade of immune responses that orchestrates a process that restores homeostasis and, thereby, limits the damage and facilitates repair. According to this view, immediately after an insult a variety of toxic mediators emerge. As a result, the local innate immune cells (microglia) are activated by the dying cells and/or by the self-compounds that exceed physiological levels and become toxic [36,37]. Thus, the surrounding still-healthy neurons are subjected to a threatening milieu that, if not corrected immediately, will affect these cells as well (a phenomenon that is known as ‘secondary degeneration’). The microglia release chemokines and act to clear the damaged site from the debris and toxic self-compounds. Subsequently, antigens released from the damaged tissue are carried to the draining lymph nodes by local APCs, which in turn activate T cells that specifically recognize self-antigens released at the damaged site [38,39]. Importantly, such self-antigens by themselves are not necessarily pathogenic, as is the case of neoantigens in tumors. The CNS-specific T cells home to the damaged site, where they engage in cross talk with local APCs (such as microglia and infiltrating macrophages) [40]. As a result of this T-cell–APC interaction, cytokines and chemokines are released from both the T cells and the APCs, inducing an infiltration of a second wave of bone-marrow-derived monocytes. These monocytes, which are now exposed to the T-cell-regulated immunological milieu at the site of injury, produce growth factors, such as IGF-I and BDNF, that contribute to neuronal survival (i.e. preventing spread of damage) and to tissue repair by endogenous stem or progenitor cells [41,42]. This series of events, which occurs after CNS insult or deviation from homeostasis, might represent an elimination phase analogous to the one observed in tumor immunology. By nature acute insults in the CNS result in a steady state; a scar tissue composed of glial cells and extracellular matrix proteoglycans (e.g. CSPG) delineate the site of injury, whereas spared cells and newly formed neurons and glial cells reside at the margin of the quarantined injury site [43]. Thus, as far as immune system activity is concerned, acute insults are resolved at the elimination phase.

Equilibrium

We suggest that in cases of chronic neuropathological conditions, the failure to completely eliminate the threat and restore homeostasis leads to conditions that appear similar to those found in the equilibrium phase of the immune response against tumors; during this phase the disease is dormant (that is, symptom free). Such situations might occur in chronic neurodegenerative disorders such as Alzheimer’s disease, Parkinson’s disease and ALS. Although animal studies have shown that in all these pathologies, once the clinical symptoms emerge, immune activity affects the course of the disease [44–46], we suggest that the immune system struggles with early manifestations of these diseases long before they become symptomatic. In this way immune activity could maintain neuropathological disorders in a dormant state.
for years – very much like it does in cancer. The point at which clinical symptoms appear represents the beginning of what could be considered as the parallel to the ‘escape’ phase. This escape phase could be an outcome of suppression of the immune response imposed by the dying neurons or an outcome of a local innate inflammatory response, as will be discussed below. The proposed relationship between disease onset and immune activity is shown schematically in Figure 1.

**Escape**

In contrast with tumor immunoediting, in neurodegenerative disorders the immune system does not impose true selection forces on the factor(s) that induce the damage. This distinction is integral to the fact that in cancer immune activity is required to selectively kill cells, whereas in neurodegeneration immune activity is needed to remove the emerging threats and to promote cell survival and renewal in a nonselective manner. Nevertheless, during the course of a neurodegenerative disease, toxicity mediators, damaging factors and dying cells can escape immune surveillance

**How ‘escape’ explains emergence of neurodegenerative diseases**

As in tumor escape suppression of adaptive immunity and overwhelming local inflammation both can lead to escalation of neurodegenerative processes (Figure 1). Accumulation of amyloid beta (Aβ) deposits (amyloid plaques) is one of the hallmarks of Alzheimer’s disease. It is a slow process that takes place years before clinical signs of the disease appear. Although the exact neuropathological mechanisms of this disease are yet to be defined, it is clear that amyloid deposition has a central role in the ensuing degenerative process. The association between accumulation and activation of microglia – the brain’s resident innate immune cells – and plaque formation has been known for years [47,48]. However, the exact role of microglia in the Alzheimer’s-affected brain has not been clear. On the one hand microglia can restrict plaque formation by secreting proteolytic enzymes such as metalloproteinases [49] and the endopeptidase neprilysin [50]. Microglia also are able to clear Aβ by receptor-mediated phagocytosis [51]. On the other hand microglia are thought to aggravate the degenerative process by producing various proinflammatory cytokines [52].

A recent study elucidated a role for the microglial CC-chemokine receptor-2 (CCR-2) in mediating microglial accumulation in the brains of transgenic mice that express the human amyloid precursor protein (APP) and develop an Alzheimer’s-like pathology [53]. APP transgenic mice that were deficient in CCR-2 exhibited increased mortality and Aβ accumulation, which was correlated with impaired microglial accumulation. CCR-2-dependent microglial accumulation was found to begin before Aβ plaques were detectable, suggesting that microglia are sensitive to early neurotoxic effects of Aβ or to conditions that lead to Aβ formation [53]. Elevated levels of monocyte chemotactic protein-1 (MCP-1), the ligand of CCR-2, observed in the brains of Alzheimer’s disease patients [54] support the possible involvement of CCR-2 in the neuroprotective immune response. This is, however, not always the case. Animal studies have shown that overexpression of MCP-1 desensitizes CCR-2 on microglia, rendering them less responsive to damage-induced chemotraction and accumulation [55]. Thus, chronic elevation in MCP-1 also could facilitate an escape phase from immune surveillance in Alzheimer’s.

Another important issue is the origin of the microglial cells that fight neurodegeneration. This point was addressed by the generation of chimeric mice in which the bone marrow of Alzheimer’s disease transgenic mice was replaced with bone marrow from transgenic mice that express green florescent protein (GFP) [56,57]. These studies have shown that bone-marrow-derived GFP+ microglia (which perhaps should be better referred to as myeloid cells rather than microglia) are the cells that remove Aβ, whereas brain-resident microglia are ineffective in Aβ

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**Figure 1.** Immunoediting in neurodegenerative disease. The model, based on the proposed phases of tumor immunoediting, depicts the levels of immune activity and clinical symptoms as a function of neurodegenerative disease progression. Basal levels of CNS immune surveillance take place constantly. Deviation from homeostasis (e.g. due to injury or accumulation of toxic self-compounds) recruits immune activity, which either acutely eliminates the damaging factors (dashed blue line; elimination phase) or continues to fight them chronically (blue line). Escape triggers the appearance and escalation of clinical symptoms (red line) and can be the result of either immune deficiency (or other similar phenotypes, such as tolerance or anergy) or uncontrolled inflammation. Notably, the rate of disease progression during the escape phase can be influenced by a number of host factors. For example, patients who have more years of education are more likely to be able to cope with early Alzheimer’s disease brain pathology without observable deficits in cognition [91].
From our current perspective this observation suggests that one of the key damage mediators is the escape of injured CNS tissue from immune surveillance by the brain’s resident immune cells. It is possible that such an escape could be in the form of ignoring the threat or overactivation, leading to uncontrolled inflammation. Bone-marrow-derived microglia or myeloid cells eliminate Aβ by enzymatic degradation or phagocytosis, but the exposure to Aβ seems to exert a price from these cells. Aβ acts through CD14 and the pattern recognition receptor TLR4, and causes microglia to develop an inflammatory phenotype characterized by elevated expression of proinflammatory cytokines such as IL-1β, TNF-α and IL-6. The role of inflammation in exacerbating the outcome of neurodegenerative diseases has been discussed in detail previously [47,48,58]. From the perspective of brain immune surveillance, uncontrolled inflammation can serve as a mechanism by which immune surveillance loses control of degenerative processes. In line with this notion, there are numerous reports demonstrating the role of inflammation in promoting tumor growth [59,60].

Another neurodegenerative disease in which escape from immune surveillance could take place is ALS, a neurodegenerative disease that predominantly affects motor neurons. Most of the knowledge about pathophysiological mechanisms of ALS derives from experiments done in a strain of transgenic mice that spontaneously develops an ALS-like disease. These mice express the mutant human Cu²⁺/Zn²⁺ superoxide dismutase (SOD1) protein, which corresponds to 10% to 15% of the familial ALS cases (i.e. with a genetic basis). Familial ALS represents only 5% to 10% of all cases. Most cases of ALS, however, are sporadic and have unknown causative factors [61]. Although extensive studies have been performed on the ALS mice, it is still not clear how the mutant SOD1 causes specific motor neuron degeneration. It is clear, however, that the mutant SOD1, which is ubiquitously expressed in all tissues, does not exclusively affect motor neurons. One possible mechanism for an escape from immune-mediated maintenance in ALS might be in either the elimination or the equilibrium phases, at the stages of the clearance of the apoptotic motor neurons. Unlike phagocytosis of pathogens, which induces a strong immune response, engulfment of apoptotic cells usually elicits a mild anti-inflammatory response or tolerance [60]. Such an immune response is characterized by secretion of TGF-β and IL-10 [62,63]. If motor neurons die in ALS by apoptosis [64], similar to the cell-elimination process that occurs during neuronal development, such cell death would not activate the body’s surveillance systems. The possibility that an apoptosis-modulated immune response contributes to immune escape in ALS is supported by the observations that in ALS animal models, as in patients with ALS, serum TGF-β levels increase as the disease progresses [65,66]. Indeed, these findings fit well with the suggestion that tumor-derived TGF-β might help to promote tumor escape from immune surveillance [67].

In support for a role of immune cells in ALS disease progression are several studies showing that replacing the bone marrow of ALS mice with bone marrow derived from healthy animals increases life expectancy [56,68,69]. An elegant demonstration of the effect of CNS-resident microglia in ALS disease progression comes from an experiment in which bone marrow from wild-type mice was transplanted into neonatal ALS mice, which also suffer from a complete immune deficiency [45]. In these mice the neonatal bone marrow transplantation resulted in population of the brain with microglia that did not express the mutant SOD1 form. This manipulation slowed motor neuron loss and prolonged disease duration and survival when compared with mice receiving bone marrow transplantation from ALS mice (i.e. containing the mutant SOD1). Importantly, transplantation of bone marrow from ALS mice into wild-type mice did not induce any signs of neurodegeneration. This study confirmed that microglia are affected by the SOD1 mutation in a way that causes exacerbation of the disease but that they are not the primary damaging components.

The majority of studies suggest that microglia contribute to ALS progression by producing toxic inflammatory compounds. In vitro studies have shown that microglia from ALS mice produce higher levels of TNF-α when stimulated with LPS compared with wild-type microglia. A recent study found that mutant, but not wild-type, SOD1 is released from motor neurons and can, by itself, activate microglia so as to become detrimental [70]. Collectively, the findings from ALS mice suggest that escape from immune surveillance can be achieved, at least in part, through alteration of the microglial phenotype. Microglial activation has been demonstrated in the brain and spinal cord of ALS patients and in the spinal cord of ALS mice. Moreover, relative to wild-type mice, elevated levels of MCP-1 were found in ALS mice as early as 15 days of age; by 39 days of age, CD68⁺ cells (presumably dendritic cells) were found in the spinal cord of ALS mice [71]. These findings suggest that the damage begins to develop very early in life, long before clinical signs are manifested. Yet, although some signs of immune activity are evident before the paralyzing symptoms appear, significant infiltration of bone-marrow-derived monocytes and T cells only occurs at very late stages of the disease (G. Kunis et al., unpublished), suggesting that the death of the motor neurons either is not sufficient to trigger the adaptive immune response that is required for the recruitment of peripheral myeloid-derived cells needed for defense or that this response is actively suppressed. We are proposing that immunoediting processes with distinctive escape mechanisms take place in both Alzheimer’s disease and ALS.

**Therapy**

The studies reviewed here suggest that immunoediting mechanisms exist not only in cancer but also in neurodegenerative diseases. If physiological, naturally occurring immune activity fails to eliminate a neurodegenerative process, the struggle between immune activity and disease-causing factors proceeds to the equilibrium phase. Thus, ideally, therapeutic interventions should take place during the equilibrium phase in order to strengthen the body’s own repair mechanisms and prevent the escape phase and the subsequent onset of clinical symptoms. This approach currently is not feasible because of the lack of measurable markers for the equilibrium phase; presently...
neurodegenerative diseases are diagnosed only after symptom emergence. Possible therapeutic interventions, therefore, could aim to recruit a beneficial immune response soon after clinical symptoms appear. This can be achieved by modulating immune activity to enhance the response to abnormal cells, to apoptotically dying cells or to damage-inducing self-compounds. However, such therapeutic modalities also must prevent the occurrence of an unregulated inflammatory response. Boosting autoimmunity by vaccination can be efficient in ameliorating neurodegenerative conditions [72,73]. Yet, if the choices of the antigen, regimen, carrier and timing of immunization are not made carefully, a deleterious effect is imposed [41,72,74]. However, because of the prevailing view that all neurodegenerative diseases involve a local inflammatory response, most approaches to therapy have attempted to tame immune activity by using anti-inflammatory drugs. For example, attempts have been made to use the tetracycline derivative minocycline to treat ALS and Alzheimer’s disease. In animal models minocycline attenuates neuronal cell death and partially reverses cognitive impairment in Alzheimer’s disease and ALS models [75–77] and decreases tumor metastasis [78]. In ALS patients treatment with minocycline not only failed to show any benefit but also was even associated with some disease exacerbation and adverse side effects [79–81]. These results indicate that in ALS, minocycline-mediated blockage of inflammation also dampens beneficial immune activity. Similarly, in healthy adult animals chronic minocycline treatment results in impaired neural cell renewal [1].

Other types of anti-inflammatory drugs that are being considered for treating these diseases belong to the family of non-steroidal anti-inflammatory drugs (NSAIDs), such as the nonselective cyclo-oxygenase (COX) inhibitors ibuprofen and indomethacin and the COX-2-selective inhibitors. NSAIDs have been regarded as promising candidates for treating Alzheimer’s (reviewed in Ref. [82]) and ALS [83], as well as other neurodegenerative diseases [84]. However, based on our view of the role of the immune system during CNS pathology, it is possible that NSAIDs also suppress the beneficial effects of the immune activity.

We believe that overcoming immune escape should be based on controlled activation of the immune system. The choice of approach should take into consideration the nature of the immune escape suspected for each neurodegenerative disease, namely whether it is an outcome of immune suppression or uncontrolled inflammation. If the approach of choice is an active vaccination, the chosen antigen should be a weak antigen that can crossreact with the relevant self-proteins. One such compound is glatiramer acetate (GA, also known as Cop-1), an FDA-approved drug for multiple sclerosis (MS) that acts as a weak agonist of a wide variety of self-antigens expressed in the CNS. Our laboratory demonstrated that GA could be used (with or without an adjuvant) to promote an immune-mediated neuroprotective response in various models of acute CNS injury [85,86]. For chronic conditions the choice of the route and regimen of GA administration is crucially dependent on the type of disorder. For example, injection of GA emulsified in CFA was shown to prolong life expectancy of ALS mice [87] and increase neuronal survival in other animal models of neurodegenerative diseases (e.g. glaucoma [88]). Yet, adjuvant-free GA is effective in animal models of Alzheimer’s disease and glaucoma in a weekly regimen [88,89]. However, the same regimen failed to show any benefit in ALS mice (M. Schwartz et al., unpublished). A negative effect was found when the frequency of its administration was increased [90]. Daily injection (the current protocol for MS) of GA yielded a small beneficial effect in male ALS mice but shortened the life expectancy of female ALS mice (M. Schwartz et al., unpublished). Thus, in the case of ALS, further studies are required to find an optimal immunization protocol that can correctly boost immune surveillance. We propose that in order to reach this target, additional research should be devoted to the identification of possible mechanisms that might contribute to immune evasion in this disease.

Concluding remarks
Over the last decade studies in two unconnected fields, cancer and neurodegeneration, revealed that the immune system has a key role in affecting disease emergence and progression. The data reviewed here suggest that the concepts of tumor immunoeediting [8] and neuroprotective autoimmunity [4] converge; similar immune-mediated mechanisms participate in tumor elimination as in CNS maintenance and repair. Escape from these immunological surveillance mechanisms marks the victory of the disease over the body’s natural defense system. Inflammation, an extreme manifestation of poorly controlled immune activity, recently has been acknowledged as a central mechanism of escape. We propose that blocking inflammation using the pharmacological arsenal available today might be problematic because it will dampen any remaining beneficial immune activity that could still restrict disease progression while at the same time preventing immune surveillance of other systems in the body. Thus, paradoxically, the ultimate immune-based therapy will be characterized by both inflammation-restricting and auto-immune-boosting properties.

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