Extending observations on the immunogenicity of neo-antigens that arise in the course of oncogenesis and tumor progression, we suggest that somatic mutations affecting normal tissues also lead to generation of new epitopes. We hypothesize that, at least under inflammatory conditions, immune responses against such neo-antigens may lead to the elimination or functional impairment of normal cells, thus contributing to aging.

Introduction

The rate of spontaneous somatic mutations that persist after DNA repair has been calculated to be in the range of $10^{-9}$–$10^{-5}$ mutations per base-pair per cell division. In the germline, such mutations generate genetic polymorphisms, which are the substrate for natural selection. As such, spontaneous germline mutations are necessary for evolution and the preservation of life itself. Nevertheless, spontaneous mutations, even excluding those that lead to severe developmental or functional defects, do not come without a cost for the organism. Somatic mutations are indeed among the primary causes of cancer, and the progressive accumulation of mutations (and damage) with age can obviously result in the loss of structure and/or function of affected polypeptides.

In principle, the expression of mutated proteins should invoke an immune response, in particular an adaptive response involving T cells and antibodies. The adaptive immune system is indeed capable of recognizing a nearly unlimited array of antigens that may differ from each other by as little as a single amino acid. Thus, a fraction of spontaneous somatic mutations is expected to generate neo-antigens that may be recognized as non-self. Somatic cells accumulate thousands of such mutation-generated neo-antigens. In particular, cancer cells (which can be viewed as a special type of somatic cells) have been estimated to accumulate hundreds of thousands of somatic mutations. There is now abundant evidence on the elicitation of immune responses against some of the neo-antigens expressed by cancer cells. Sometimes this response is sufficient to eliminate nascent tumors, while most often it is relatively inefficient and persists as the tumor progresses. Thus, somatic mutations can generate neo-antigens that are capable of eliciting cellular immune responses. Such an immune response significantly influences tumor progression, either leading to the eradication of neoplastic cells or modulating their phenotypic and functional properties (immunoediting).

Hypothesis

Based on these premises, we postulate that an immune response is elicited against non-transformed cells bearing neo-antigens that arise by spontaneous mutations. By analogy with what occurs for cancer cells, the result of this response would be the eradication of the cells bearing such neo-antigens, or the modulation of their function. At least potentially, both these effects...
might compromise, or at least decrease, the functional reserve of a tissue. For instance, in its lifetime a stem cell is expected to accumulate thousands of neo-antigens and to pass them on to its progeny, virtually creating a tumor-like clone. The immune response could, in principle, destroy such clone and hence limit the functional reserve of the tissue/organ involved. In summary, the hypothesis can be simplified as (Fig. 1):

1) Somatic mutations accumulate throughout the lifetime of an organism, and may result in malignant transformation or other defects.

2) Such mutations, if affecting the coding regions and non-synonymous, give rise to mutated proteins that are normally degraded into peptides, which are presented in complex with MHC molecules on the cell surface as neo-antigenic peptides. In the case of cancer cells, the immune response to these neo-antigenic peptides has been shown to underlie tumor eradication or immunoeediting.8

3) Along similar lines, an immune response to neo-antigens expressed by non-transformed cells can also lead to their elimination or editing. Because of the breadth of accumulation of somatic mutations, at least potentially this may lead to a massive immune response (catastrophic autoimmunity). It must be recognized that such neo-antigens may be immunogenic11 or tolerogenic.12

4) To prevent a massive response against non-transformed tissues that express neo-antigens, various mechanisms that locally modulate or inhibit the immune system have evolved. The very same mechanisms that are responsible for such a physiological modulation (which suppress autoimmunity and, by our suggestion, prevent premature aging) also limit the effectiveness of endogenous defenses against cancer.

From this perspective, cancer is not simply a consequence of longevity, but rather is part of the price that we pay for it. Put even more broadly, the physiological mechanisms that prevent or modulate autoimmunity, and hence prolong organismal health span, are utilized by malignant cells as a defense against immunosurveillance.

**Predictions of the Hypothesis That Can be Assessed Experimentally**

A number of strategies can be used to test distinct components of our hypothesis. We suggest here 3 among the most direct methods to test its central tenets.

1) Skin grafts have been used effectively to identify minor histocompatibility (H) antigens.12 The neo-antigens discussed here can essentially be considered as minor H antigens, with some important caveats. Indeed, while all cells of a tissue would express the same minor H antigens, the neo-antigens that accumulate with aging are expressed only by a proportion of the cells in a given tissue. Furthermore, while minor H antigens are shared and stable, i.e., all mice of a given strain express the same minor H antigens, aging-related neo-antigens would be unique in each individual. The experimental strategy to reveal such neo-antigens would involve the transplantation of skin from aged mice to syngeneic young mice, followed by assessment of graft rejection. Grafts from young mice to syngeneic young mice would provide negative control conditions. Such “heterochronic” skin grafts have previously been attempted, and preliminary evidence indicates that they indeed are rejected.13 Ironically, the authors of this study ruled out immunological mechanisms because the mice were of the same inbred strain! Re-grafting previously grafted young mice with tissue from the same or a different old mouse would help to determine whether or not neo-antigens are unique to individual animals.

2) Current high-throughput DNA sequencing technologies allow for directly testing our hypothesis. Deep sequencing the exomes of young and progressively older mice should indeed reveal the accumulation of mutations as a function of age. There are methodological constraints in this strategy that need to be kept in mind. Since somatic mutations affect only a fraction of the cells in a given tissue, and since different cells may have unique sets of mutations, such a sequencing approach would have to be qualitatively deeper than that used for routine sequencing. This is somewhat problematic because the error rate associated with current deep sequencing methods may indeed be higher than the rate of spontaneous mutations in some non-transformed tissues. Thus, such a direct approach might have to wait until the technology that underlies deep sequencing has achieved a higher accuracy. Interestingly, Stringer et al.4,5 have elegantly demonstrated the existence of spontaneous mutations in non-transformed tissues from aged mice. To do so, they generated transgenic mice harboring an enzyme that is not actively synthesized owing to an upstream frameshift mutation. By this approach, they were able to estimate the rate of spontaneous mutations based on those that restored the enzymatic activity.

3) The studies by Stringer et al.4,5 suggest new avenues to explore the other central tenet of our hypothesis, i.e., the elicitation of an immune response against neo-antigens expressed by non-malignant cells. In particular, mice could be engineered to carry immunogenic peptides that are expressed only upon mutational events that productively reverse an upstream frameshift mutation. In this setting, the immune response against such peptides could be monitored in real time (with specific tetramers) throughout the lifespan of individual mice.

**Cancer and Aging: A Faustian Proposition**

The expression of new antigens arising upon spontaneous somatic mutations should be so universal that one might ask why old organisms do not succumb to massive autoimmune responses? We take a leaf from our understanding of anticancer immune responses to explain this apparent paradox. It has become clear that tumors have a variety of strategies to evade the immune response. Among other, these include the secretion of factors that interfere with the activation of the immune system (e.g., transforming growth factor β1) and the expression of molecules that down regulate the immune response, such as B7-H1, signal transducer and activator of transcription 3 (STAT3), and adenosine.14 We postulate that these strategies are not a manifestation of the malignant process per se, but rather are normally in place to prevent the autoimmune catastrophe that
would result from the responses to neo-antigens expressed by non-transformed cells. In the absence of such mechanisms, the immune system might be able to prevent all cancers, yet would radically shorten the health span of the host. Thus, the mechanisms designed to protect non-transformed cells expressing mutated polypeptides from destruction are co-opted by neoplastic cells. This idea is not to deny the existence of other mechanisms specifically activated by cancer cells to avoid immune recognition, but simply to suggest that mechanisms used by normal cells may suffice to this aim.

Our view, as framed in the previous section, raises another question: “What determines which tumors or normal tissues will be effectively targeted by the immune system?” The experience from anticancer immune responses may again be instructive. A developing neoplasm that expresses tumor-associated antigens may continue to grow in an immunocompetent host or may be rejected. One of the factors that determine the outcome (tumor growth or rejection) of the interaction between malignant cells and the immune system has been termed immunological ignorance. It appears that a cancer expressing highly immunogenic antigens can continue to grow because it can subvert the host immune response (via immunosuppression or immunological tolerance) or because the immune system simply ignores tumor-associated...

**Figure 1.** Essential elements of our hypothesis. (A) Normal tissue with no mutations and no neo-antigens. (B) Cells within the tissue undergo random somatic mutations, a proportion of which (~2/3rd) are expected to be non-synonymous. A fraction of such non-synonymous mutations can lead to immunologically recognizable MHC-restricted neo-epitopes (indicated by nuclei of different colors). The cartoon exaggerates the frequency of the neo-epitopes, which is obviously very low and varies with tissue type based on the spontaneous mutation frequency of constituting cells. (C) The cells harboring the neo-epitopes (as well as other cells) expand along with the turnover of the tissue. This expansion may result from the asymmetric proliferation of stem cells or more differentiated tissue precursors. (D) The immune system may remain ignorant (or tolerant) of the neo-epitopes, except in case of an infection, trauma or other event that may create an inflammatory environment and/or local necrosis. (E) Antigen-presenting cells (APCs) infiltrate the tissue, engulf pathogen-encoded antigens (if any) as well as the neo-antigens, and initiate the priming of naïve T cells against these epitopes. (F) Primed effector T cells eventually infiltrate the tissue and attack pathogen-infected cells (if any) as well as the cells expressing the neo-antigens. (G) These events result in the loss of tissue mass due to the elimination of parenchymal cells by T lymphocytes. The resulting damage might be limited by immunosuppressive mechanisms to prevent catastrophic autoimmunity (not shown). Nonetheless, over time this may contribute to the functional tissue impairment that is associated with aging.
antigens. Such an ignorance may be broken in the presence of specific microenvironmental conditions, resulting in the elicitation of a tumor-eradicating immune response. Conditions that “awaken” the host from immunological ignorance include the presence of local and systemic pro-inflammatory stimuli such as tumor lysis or infection. These conditions result indeed in the release of intracellular components that operate as natural adjuvants, including heat shock proteins, nuclear and mitochondrial DNA, high mobility group box 1 (HMGB1), endogenous ligands of vaniloid receptors, and possibly others. These adjuvants stimulate the activation of local antigen presenting cells (APCs), their capacity to capture of antigens present in the extracellular milieu, their maturation and their migration to draining lymph nodes. Such APCs become capable of priming tumor-specific T cells that mediate tumor rejection. Systemic inflammatory conditions such as those elicited by fever, “cytokine storm,” etc... may also mediate similar effects. This entire chain of events (which comes in many possible variations) is essential for the elicitation of a productive immune response, while the mere presence of a cellular entity harboring neo-antigens is not sufficient.

Extrapolating these lessons to non-transformed tissues bearing neo-antigens as a consequence of somatic mutations, one would hypothesize that normal tissues are ignored by the immune system until the moment in which an inflammatory event breaks immunological ignorance. An infection as well as traumatic event (e.g., a fall), both resulting in the local release of endogenous adjuvants, may thus awaken the immune system to the presence of neo-antigens on non-transformed cells. The consequent immune response may lead to the death of these cells themselves or to their functional impairment, hence contributing to aging. This series of events is consistent with the notion that most people do not age in a continuous fashion, but in discrete steps, with events such as infections and traumas providing the punctuation marks. In this scenario as well, the very same events that underlie antican immune responses contribute to aging.

Other Perspectives and Implications

The adaptive immune system is a central component of the hypothesis that we presented here. Aging, however, is not reserved to species endowed with such a system. Rather, a whole range of species that have no adaptive immune system do actually age and die. Clearly, a range of factors including (but not limited to) the attrition of telomeres, oxidative damage, and somatic mutations (even in the absence of an immune response) are sufficient to bring about the functional impairment associated with aging. The mechanisms hypothesized here must therefore be considered as contributors to the aging process rather than as its exclusive etiological determinant. This said, we suggest that aging in the presence of an adaptive immune system is qualitatively different from aging in its absence.

A recent study by Baker et al., demonstrating that the depletion of senescent cells attenuates many symptoms commonly associated with aging in mice, might appear to directly contradict a key element of our theory, i.e., the hypothesis that the loss of senescent cells contributes to the functional defects of tissues and organs accompanying aging. A closer scrutiny of the model employed in this study shows that the findings by Baker and collaborators in fact do not argue against our hypothesis. The authors used progeroid mice engineered so that the tumor suppressor p16Expressing cells could be harnessed to trigger the apoptotic demise of senescent (p16Expressing) cells every 3 d, beginning at 3 weeks of age. This resulted in a delay in age-correlated disorders. Mice that were depleted of senescent cells by this approach indeed manifested increased levels of inguinal adipose tissue, increased muscle fiber diameters, and improved exercise ability over control animals. Actually, this study makes an important point with respect to our hypothesis, as in this model senescent cells are never allowed to constitute part of the adult mouse. Hence, this model is intrinsically inapt to assess the functional consequences of the loss of senescent cells in an aging adult mouse. Furthermore, since senescent cells are never allowed to accumulate, they do not have the opportunity to secrete pro-inflammatory cytokines and to create the inflammatory environment that is required for immunological ignorance to convert into a productive immune response.

Since somatic mutations are a cornerstone of our hypothesis, clinical syndromes linked to genetic defects impacting on DNA repair should be of interest in dissecting it. Of particular interest should be a comparison of the syndromes that are associated with immunodeficiency (such as xeroderma pigmentosum) with those that are not (such as Cockayne’s syndrome). Interestingly, patients with Cockayne’s syndrome, who do not have known immunological defects, exhibit symptoms of progeria, while patients with Xeroderma pigmentosum do not. Obviously, these facts are too disparate and too broad to permit definitive conclusions. However, they do suggest that an examination of genetic defects influencing DNA repair may constitute a unique opportunity to test our hypothesis, namely, the Faustian bargain between aging and cancer.

Our hypothesis has some implications for the use of stem cells as a therapeutic agent. According to our construction, indeed, tissues (and by extension whole organisms) derived from stem cells would be different from the parental organism, based on the specific accumulation of somatic mutations. Moreover, stem cells accumulating somatic mutation might become targets of an immune response, much as organs with minor histo-incompatibility do. However, to the extent that asymmetric stem cell division preferentially leads to cell replacement, the risk that stem cell transplantation would be affected by a substantial burden of neo-antigens (and hence trigger an immune response) appears as substantially mitigated.

Although the immune responses elicited by transformed cells have been intensively studied, the immunological potential of non-transformed cells bearing somatic mutations is largely unexplored. The hypothesis presented here stems logically from what is known about antitumor...
immunity and from the general concept of non-self recognition. Further experiments, such as those suggested here, should establish whether these concepts have been appropriately applied, or whether alternative mechanisms govern the interaction between the immune system and non-transformed cells that express neo-antigens.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Acknowledgments

We thank Drs. George Kuchel and Morgan Carlson of the University of Connecticut Center on Aging, and Derry Roopenian of the Jackson Laboratory, for critically reading the manuscript.

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