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Impact of aging in cancer immunotherapy
The importance of using accurate preclinical models

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Abbreviations: FDA, Food and Drug Administration; IFNγ: interferon γ; IL, interleukin; TNFα, tumor necrosis factor α

Cancer immunotherapy holds great promise, yet its efficacy and applicability can be hampered by the rise of systemic toxicities. We have recently shown that the lethal side effects of cancer immunotherapy are markedly exacerbated with aging. Blocking tumor necrosis factor α or macrophages can alleviate the systemic toxicity of immunotherapy while preserving its antineoplastic effects.

The ability of immunotherapy to evoke successful antitumor immune responses has been well documented over the past decade. In spite of abundant preclinical data, it is only with the recent approval by the Food and Drug Administration (FDA) of drugs like sipuleucel-T and ipilimumab that immunotherapy is finally being recognized as a viable alternative to traditional therapies for treatment of various cancers.[1] However, despite the ability of immunotherapy to elicit successful antitumor immune responses, its efficacy is hindered by several factors. In particular, systemic toxicities are major obstacles in this setting and often lead to treatment interruption. Indeed, such adverse effects, which can be immunological and/or parenchymal, can be particularly severe and even fatal to some patients.

The development of new anticancer immunotherapies currently relies on the use of young mouse models. The use of young (2–4 months old) mice instead of aged mice complicates the extrapolation of results to the human setting, as cancer patients are often elderly.[2] Aging is a dynamic, irreversible process that leads to the accumulation of pathophysiological changes, including decreased/altered immunological functions over the lifetime of the individual. Since immunotherapy aims at the induction or potentiation of anticancer immune responses, it is crucial to develop new immunotherapeutic paradigms that are tailored on the elderly. Appropriate preclinical models are necessary to this aim.

Studies in mice and humans have demonstrated that aging is associated with a "low-grade pro-inflammatory state," a systemic condition characterized by aberrant cytokine production and inflammation.[3] Elevated levels of various pro-inflammatory cytokines including interleukin (IL)-1, IL-6, and tumor necrosis factor α (TNFα) have been detected in healthy, aged individuals.[4,5] Age-associated diseases (e.g., atherosclerosis, Type 2 diabetes) may actually exacerbate such a pro-inflammatory state.[6]

We have previously demonstrated that agonistic CD40-specific antibodies combined with IL-2 mediate synergistic therapeutic effects in young mice (2–4 months old) bearing metastatic tumors.[7] Because young mice are the equivalent of adolescent and young adults, we tested this immunotherapeutic regimen in middle-aged (12–15 months old) and aged (> 16 months old) mice.[8] We found that aged mice, at odds with their young counterparts, rapidly succumb to CD40-specific antibodies plus IL-2 within 2 d of administration, with several organs involved. Such an increased mortality was accompanied with elevation in pro-inflammatory cytokines including IL-6, TNFα and interferon γ (IFNγ), both systemically and in situ (i.e., in the liver, lungs, and gut). Notably, the increased secretion of IL-6, TNFα, and IFNγ in response to combinatorial immunotherapy was proportional to age. Ultimately, the systemic toxicity of immunotherapy led to multi-organ failure and death, both in aged and middle-aged mice. Of note, similar adverse effects were documented in multiple murine strains and in response to other immunomodulatory regimens, for instance the combinatorial administration of IL-2 or IL-12 and lipopolysaccharide (LPS).

We have demonstrated that the systemic toxicity of immunotherapy is independent of natural killer (NK) cells as well as $CD4^+$ and $CD8^+$ T cells, as the depletion of these cell populations prior to immunotherapy did not prevent the cytokine storm that provoked the rapid death of aged mice.[8] Conversely, the depletion of macrophages from aged mice decreased the severity of the systemic cytokine storm

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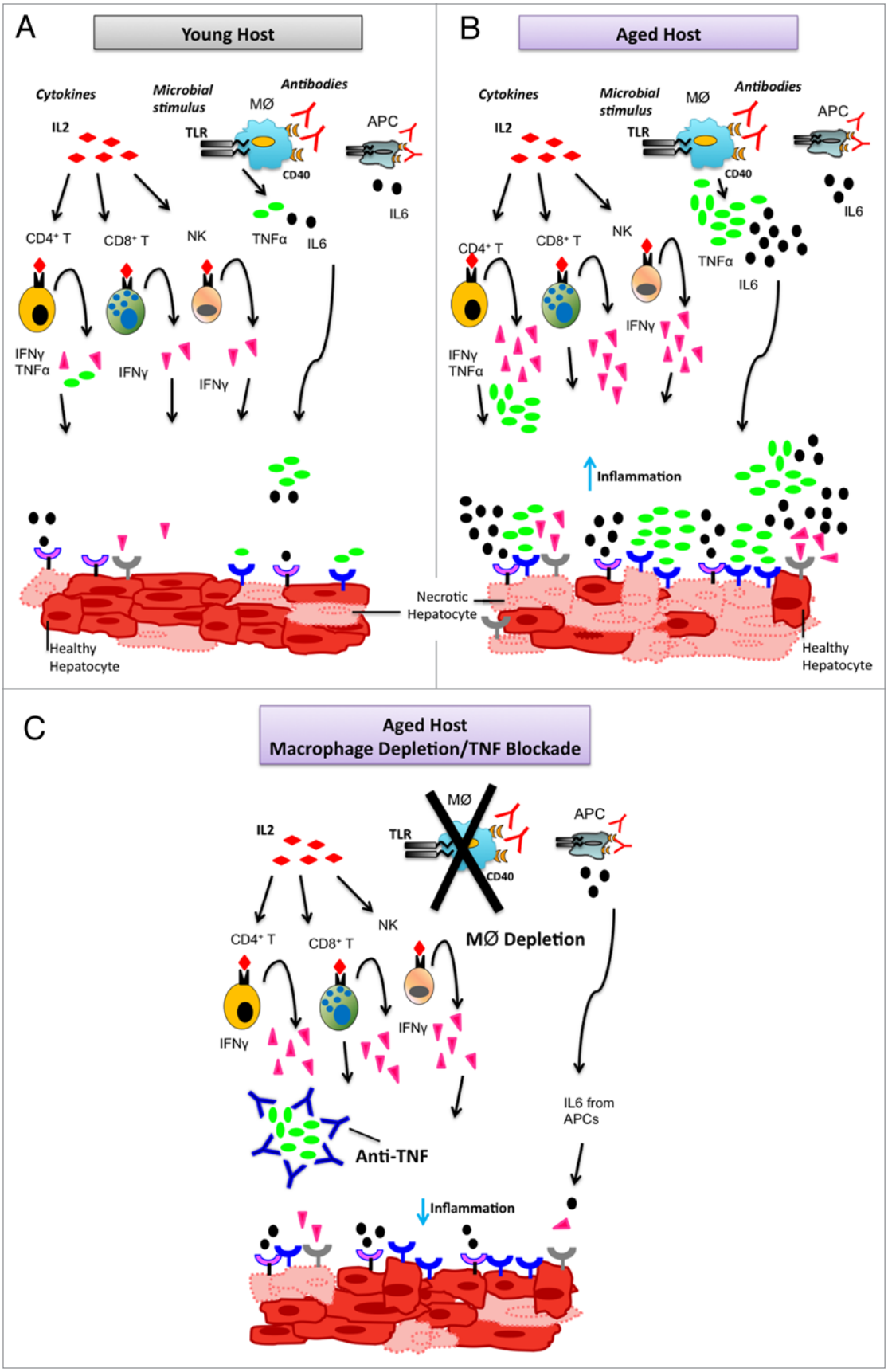

Figure 1. See figure legend on following page.

Figure 1. (See following page) Strategy to circumvent potentially lethal side effects of cancer immunotherapy in the elderly. (**A–C**) Immunomodulatory agents including cytokines, such as interleukin (IL)-2, microbial components that operate as Toll-like receptor (TLR) agonists, and immunostimulatory antibodies, such as CD40-targeting antibodies, lead to the systemic secretion of pro-inflammatory cytokines. IL-2 leads to the production of interferon γ (IFNγ) by $CD4^+$ T cells, $CD8^+$ T cells and natural killer (NK) cells, as well as to the secretion of tumor necrosis factor α (TNFα) by $CD4^+$ T cells in both young (**A**) and aged (**B**) hosts. Microbial components and immunostimulatory antibodies activate macrophages (MØ) and other antigen-presenting cells (APCs) to produce TNFα and IL-6 in both young (**A**) and aged (**B**) animals. However, the administration of immunostimulatory agents to aged hosts results in the massive secretion of pro-inflammatory cytokines, leading to multi-organ failure (**B**). Immunotherapeutic interventions result in a robust inflammatory responses and extensive hepatic necrosis in aged (**B**), but not young (**A**) mice. The neutralization of TNFα by specific antibodies or macrophage depletion limits the secretion of pro-inflammatory cytokines by aged hosts responding to immunotherapy, hence inhibiting systemic and local inflammation, reducing hepatic necrosis and ultimately protecting these animals from immunotherapy-associated lethality.

induced by immunotherapy, de facto protecting animals from the hepatotoxic effects and lethality of CD40-specific antibodies plus IL-2. To further dissect the role of macrophages in this setting, we determined the cytokine secretion profile of macrophages isolated from aged or young mice as well as from healthy volunteers. Our data show that, upon stimulation, macrophages from aged mice secrete much higher amounts of TNFα and IL-6 than macrophages from young mice.[8] Consistent with these findings, we found that the production of TNFα and IL-6 by human macrophages increases with the age of the donor, indicating that aging predisposes macrophages to acquire a pro-inflammatory phenotype.[8]

Since macrophages are major sources of TNFα and IL-6, and due to the well-known role of TNFα in inflammation,[9] we wondered whether TNFα plays a major role in the systemic toxicity of immunotherapy in aged mice. We observed that the administration of CD40-specific antibodies and IL-2 to either aged *Tnfa*$^{-/-}$ mice (which lack the TNFα–coding gene) or aged mice pre-treated with the TNF receptor-IgG1 fusion protein etanercept (which is approved by FDA for the treatment of multiple inflammatory diseases) was associated with limited increases in circulating IL-6, reduced hepatotoxicity and a significant increase in survival. Finally, because TNFα has both anti- and pro-tumor functions,[9,10] we sought to determine whether or not the blockade of TNFα in aged mice receiving immunotherapy would hamper its antineoplastic effects. Interestingly, the combination of etanercept with CD40-specific antibodies plus IL-2 preserved the cytotoxic functions of $CD8^+$ T cells, mediated robust antitumor effects and prolonged the survival of aged mice bearing lung carcinoma.[8]

In summary, our data suggest that cancer immunotherapy in the elderly is associated with an increased secretion of pro-inflammatory cytokines and an augmented susceptibility to death, mostly owing to a multi-organ toxic syndrome that is macrophage- and TNFα-dependent (Fig. 1). Since the incidence of several malignancies increases with age,[2] we believe that experimental efforts aimed at developing novel cancer immunotherapies should strongly consider aged animals as a model to obtain profound mechanistic insights into the side effects of immunotherapy. Importantly, especially from a therapeutic standpoint, immunostimulatory interventions coupled to the blockade of potentially lethal mediators (i.e., TNFα) might mediate long-lasting therapeutic effects in the absence of excessive toxicity for the patient. It will be of particular interest to investigate the role of TNFα in the toxicity of other anticancer treatments, including radio- and chemotherapy, among the elderly.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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