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an armed state. It remains to be defined, however, whether the latent infection is sufficient to enable the indefinite persistence of armed NK cells in the mouse. In the setting of *Listeria* infection, MuHV-4 infection was shown to lead to enhanced immunity for months, but not indefinitely.[10]

White et al demonstrate the arming phenomenon as a feature of latent infection in several ways. First, splenocytes from RAG$^{-/-}$ mice housed under SPF conditions demonstrated increased granzyme B protein expression 72 hours after adoptive transfer into a latently infected recipient animal. This did not require proliferation, as the transferred cells became armed but did not dilute a CFSE label. Second, the authors made use of a mutant MuHV-4 (073.stop virus), which has a genetically defined reduced capacity to establish latency. Interestingly, infection with the 073.stop virus resulted in a typical NK-cell response to acute infection with increased cytotoxicity and granzyme B expression, but failed to lead to the presence of NK cells with increased granzyme B protein after the virus had been cleared. Thus, arming as defined by granzyme B expression and increased capacity for cytotoxicity was a feature of the latent infection. This translated to increased NK-cell function and host defense, as only mice with wild-type MuHV-4 latent infection were able to survive a challenge with RMA-S tumor cells (a classical model of in vivo NK-cell activity).

The mechanism of how latent infection arms NK cells, however, was not explored and remains unclear. Latency of MuHV-4 is established in certain lymphoid tissues, thus perhaps representing a special circumstance with regard to access to the NK-cell compartment. As the authors point out, armed NK cells exist among human cord blood, suggesting that mechanisms other than direct exposure to host cells latently infected with herpesviruses are likely to exist.

The present work raises several additional questions. These include how the phenomenon of arming coexists with that of licensing, or the developmental enabling of cytotoxicity due to the appropriate exposure to regulatory signals.[11] Are the majority of armed NK cells licensed, and have they traditionally developed prior to arming? Similarly, if there is a population of armed NK cells that were not conventionally licensed, are there differences in how these cells persist over time compared with those that are licensed? Furthermore, although the authors have previously defined that arming can be regulated at the level of translation, it remains unclear how this relates to maturation and development of the complex lytic machinery itself.[2] It is likely that some lytic effector components are constitutively available and others induced in the process of arming.

Another question is how armed NK cells that develop in the context of latent infection relate to the recently described memory NK cells.[12,13] Can armed NK cells give rise to memory NK cells? There are at least some distinctions between the two, as the memory NK cells are observed after contraction of the response and demonstrate a reduced ability to kill. Thus, perhaps the armed NK cells represent a population that has never had the opportunity to enter into the memory phase due to a persisting signal for arming (see figure). This would be suggested by the experiments using the 073.stop virus, in which armed NK cells did not persist.

Although the demonstration of arming raises important questions, it does potentially bring into alignment some experimental distinctions between human and murine NK cells. It also suggests a benefit to the host of becoming latently infected with herpesvirus, where latent infection facilitates the persistent readiness of NK cells and potentially promotes NK cell–mediated surveillance against tumors, which would presumably lead to improved human survival.

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Comment on Nakahara et al, page 4384

Cyclophosphamide, DCs, and Tregs

Hironori Matsushima and Akira Takashima UNIVERSITY OF TOLEDO

Certain chemotherapeutics are now known to augment host immunity by acting on DCs. In this issue of *Blood*, Nakahara and colleagues demonstrate a unique pharmacologic activity of CTX to selectively eliminate the lymphoid tissue–resident CD8^{+} DC subset in mice.[1]

Although chemotherapeutic agents are generally believed to suppress the host immunity, recent studies have unveiled unexpected potentials of some agents to augment the adaptive immune responses against cancer cells (see table). For example, anthracins (eg, doxorubicin and mitoxantrone), but not other DNA-damaging drugs, induce an immunogenic form of cancer cell death characterized by surface expression of calreticulin, which in turn promotes efficient phagocytosis of dying cancer cells by dendritic cells (DCs).[2] Cancer

Drugs	Target cells	Direct effects	Changes in DCs
Anthracins[2]	Cancer cells	Calreticulin surface expression	Enhanced phagocytosis of dying cancer cells
Doxorubicin[3]	Cancer cells	HMGB1 secretion	Enhanced antigen processing and cross-presentation
Bortezomib[4]	Cancer cells	HSP90 surface expression	Augmented ability to stimulate tumor-specific T cells
Vinblastine[5]	DCs	DC maturation	Augmented T cell-stimulatory activity Enhanced antigen cross-presentation
Cyclophosphamide[1]	DCs	Selective depletion of $CD8^+$ DCs	Augmented T cell-stimulatory activity accompanied by reduction in Treg number and function

DC stimulatory properties of selected chemotherapeutic agents.

cells being killed by doxorubicin also secrete the high-mobility-group box 1 (HMGB1), which augments antigen processing and presentation by DCs in a TLR4-dependent manner.[3] Similarly, bortezomib, an inhibitor of 26S proteasome, induces surface expression of heat shock protein 90 (HSP90) on cancer cells, thereby inducing DC maturation indirectly.[4] Through unbiased screening of a relatively large number of chemotherapeutic drugs, vinblastin was found to directly trigger phenotypic and functional maturation of DCs.[5] Although cyclophosphamide (CTX) is reported in the literature to potentiate adaptive immune responses against established tumors, at least in part by abrogating regulatory T cells, (Tregs),[6,7] the underlying mechanisms responsible still remain unclear.

In the current study, Nakahara et al tested the impact of single administration of CTX on 3 distinct DC subsets in skin-draining lymph nodes in mice: (1) DCs derived from Langerhans cells and dermal DCs, termed the migratory DCs; (2) lymphoid tissue-resident $CD8^+$ DCs; and (3) plasmacytoid DCs (pDCs).[1] Although the numbers of all 3 DC subsets were significantly reduced by CTX, the $CD8^+$ resident DC subset was almost completely depleted within 4 days after CTX treatment. All DC subsets eventually returned to the baseline levels. The resulting transient imbalance in DC subsets, in turn, led to enhancement of the T cell–stimulatory function of the DC populations as a whole, suggesting the immunoinhibitory potential of the $CD8^+$ DC subset. In the in vitro antigen presentation assays, crude DC preparations isolated from CTX-injected mice induced marked expansion of a Treg population with an unusual property of interferon-γ (IFNγ) production. This effect of CTX treatment was reversed by adding the purified $CD8^+$ DC population back to the assays. Moreover, those IFNγ-producing Tregs that had been expanded with DCs from CTX-injected mice exhibited functional impairment in their in vitro ability to suppress the growth of conventional T cells.

In vivo relevance of these findings was elegantly demonstrated in mice using implantable tumor models. When adoptively transferred into tumor-bearing mice, CD8 T cells expressing transgenic T-cell receptors for a specific tumor-associated antigen exhibited clonal expansion and IFNγ production. Importantly, CTX administration further augmented such CD8 T cell responses, and this in vivo effect of CTX was abrogated by intradermal injection of $CD8^+$, but not $CD8^-$, DC preparations. In a different set of experiments, CTX administration was found to promote the rejection of tumors upon second tumor challenge, and this effect was also diminished significantly by injection of $CD8^+$ DCs, but not $CD8^-$ DCs. Thus, it appears reasonable to conclude that selective and transient depletion of the $CD8^+$ DC subset accounts for the augmented immune status observed after CTX treatment.

It is now evident that different DC subsets play distinct functional roles. When activated with pathogenic stimuli, $CD8^+$ resident DCs are fully capable of capturing and presenting various forms of exogenous antigens. In the steady state, however, $CD8^+$ DCs serve as regulatory DCs maintaining the peripheral tolerance via preferential activation of Tregs.[8] Thus, selective elimination of the $CD8^+$ resident DC population may produce opposing immunologic outcomes depending on the microenvironment in the lymphoid tissues. In the absence of pathogenic signals, CTX-induced depletion of immature $CD8^+$ DCs may augment host immune responses against cancer cells, as has been described here. On the other hand, patients receiving CTX treatments may fail to mount protective immunity against infectious pathogens due to the deficiency in mature $CD8^+$ DCs.

The study by Nakahara et al was not designed to elucidate mechanisms by which CTX impairs the balance among the tested DC subsets. CTX may have killed the $CD8^+$ resident DC subset selectively. Alternatively, CTX may have induced preferential expansion of the migratory DC and pDC subsets. In this regard, Salem et al reported that bone marrow cells isolated from CTX-treated mice produced higher numbers of DCs in culture[9] and that CTX administration augmented in situ proliferation of DCs in the bone marrow during the early phase and then in the blood and spleen during the recovery phase.[10] Likewise, working with a hepatic tumor metastasis model, Radojcic et al found that CTX administration elevated the number of tumor-infiltrating DCs in mice by promoting the rebound expansion of DC progenitors.[11] The observed imbalance may have been simply caused by the difference in turnover among the tested DC subsets—$CD8^+$ resident DCs are regarded as one of the DC subsets showing the most rapid rate of turnover.[8] Obviously, further studies are required to determine the underlying mechanisms. Nevertheless, the current study provides a new conceptual basis for the use of CTX as an "immuno-stimulatory" chemotherapeutic drug.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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Comment on Cavassani et al, page 4403

Do Tregs link sepsis to tumor growth?

Alex G. Cuenca and Lyle L. Moldawer UNIVERSITY OF FLORIDA

Sepsis has been shown to induce an immune suppressive state that includes the expansion of $CD25^+CD4^+$ $FoxP3^+$ T regulatory cells (Tregs). In this issue of *Blood,* Cavassani and colleagues demonstrate that Treg expansion in the postseptic period contributes to increased tumor growth.

The immune suppressive state associated with severe sepsis is easily recognized, but still remains poorly described. The late Roger Bone coined the term, the "compensatory anti-inflammatory response syndrome (CARS)" to reflect what he proposed was a reactive response to an exaggerated early innate immune activation and inflammation.[1] Bone also coined "immunoparalysis," which refers to the postsepsis response that is thought to not only impair resolution of the initial infection, but also to predispose the septic patient to secondary nosocomial infections.[1,2] In this issue of *Blood*, Cavassani et al provide evocative data suggesting that sepsis and its associated alterations in immune function may not only reduce immune surveillance but also contribute to more rapid growth of established solid tumors.[3] Although there are a plethora of immunosuppressive mechanisms that have been described during the postsepsis period, the authors focus their investigation on regulatory T-cell populations (Tregs).

Is this a reasonable suggestion? Sepsis-induced immune suppression is clearly multifactoral, but there is increasing evidence that sepsis is associated with the expansion of several suppressor cell populations, including both Tregs and the more newly described myeloid derived suppressor cells (MDSCs).[4,5] Importantly, there is no disagreement that the relative numbers of these cells expand during sepsis, a finding first reported by Monneret et al nearly 10 years ago.[6] Where there is still disagreement in the sepsis field is whether outcome to sepsis is dependent on Tregs and their suppressor cell activities. For example, we demonstrated several years ago that both the proportion and the suppressor activity of $CD4^+CD25^+FoxP3^+$ T cells are increased in sepsis, very similar to the results reported here by Cavassani.[5] Despite these findings, neither we nor Ayala and colleagues were able to link this increase in Treg suppressor function with increased mortality during sepsis.[5,7] Additionally, Carrigan et al were unable to show any difference in mortality to *Pseudomonas* pneumonia when Tregs were depleted.[8] To make matters more confusing, Chen and Oppenheim reported the complete opposite finding—that depleting Tregs in sepsis improved outcome.[9]

Despite the lack of consensus on the role of Tregs in sepsis, Cavassani et al in this issue of *Blood* demonstrate that the Treg expansion that occurs after sepsis results in the more rapid growth of a solid transplantable tumor.[3] The concept that Tregs can regulate tumor growth is not necessarily novel, although the linking of sepsis and Treg expansion with tumor growth clearly is. When it comes to suppressor cells and tumor growth and metastasis, considerable research in the last decade has been centered on both Treg and MDSC populations.[10] Only recently, though, has there been evidence that the MDSC population with suppressor cell function is increased in sepsis.[4]

Although the findings are convincing that Tregs play a role in the accelerated growth of established tumors after sepsis, the study raises a number of evocative questions. Unfortunately, the studies do not answer 2 inevitable questions: through what mechanisms do Tregs permit more rapid growth of an established solid tumor, and are Tregs the sole or primary agents of this response?

For the former question, the authors did report decreases in $CD8^+$ IFN-γ and perforin expression in the draining lymph nodes of postseptic and control non–tumor-bearing and tumor-bearing mice.[3] Unfortunately, the data are not antigen specific, but they do suggest that postseptic Tregs may be better at suppressing $CD8^+$ T-cell responses than those Tregs isolated from control mice, which would not be a surprising finding.[5] As $CD8^+$ T cells are important in immune surveillance and to strengthen the authors argument that postseptic Tregs may be more potent at suppressing $CD8^+$ T-cell responses than control animals, Cavassani et al adoptively transferred Tregs from postseptic animals or control animals along with naive $CD8^+$ T cells in $RAG^{-/-}$ hosts.[3] As demonstrated in the report, tumors implanted in mice that were adoptively transferred with postseptic Tregs and naive $CD8^+$ T cells were significantly larger than those tumors from control mice.[3]

The second question is much more problematic given the plethora of changes in both innate and adaptive immunity during sepsis. The challenge in studying the impact of postsepsis immune suppression on solid tumor growth is the same challenge as studying sepsis immune suppression: how to understand the role of a single mediator or cell process in the context of a complex syndrome with aberrations in multiple suppressor cell and effector cell populations. The take home message that the authors wish to convey is that the expansion of Tregs and their increased suppressor activity during sepsis may create an immune suppressive environment that accelerates the growth of a solid transplantable tumor. These conclusions are well supported by their data, and the hypothesis provides a novel research direction for Tregs in the potentiation of cancer growth and inhibition of immune surveillance in the postseptic period. However, more conclusive mechanistic data will be needed to prove that these Tregs are indeed influencing the function of $CD8^+$ T cells. In addition, as the authors themselves state, it is unknown what effect these postseptic Tregs are having on other cells known to have a role in tumor growth, such as NK cells.[3] Also, the role of MDSCs and the interplay of these 2 strongly $CD8^+$ T-cell immunosuppressive cell types need to be taken into account as both of these

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