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Edited by

George F. Vande Woude
George Klein



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Volume 107

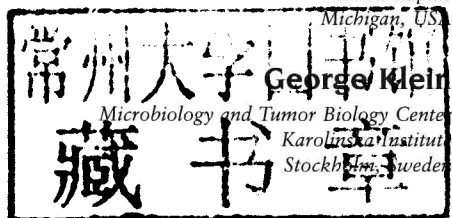
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Advances in
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Volume 107

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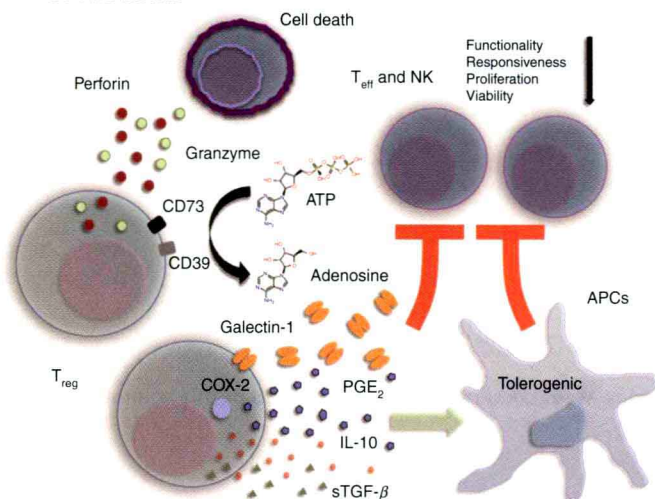
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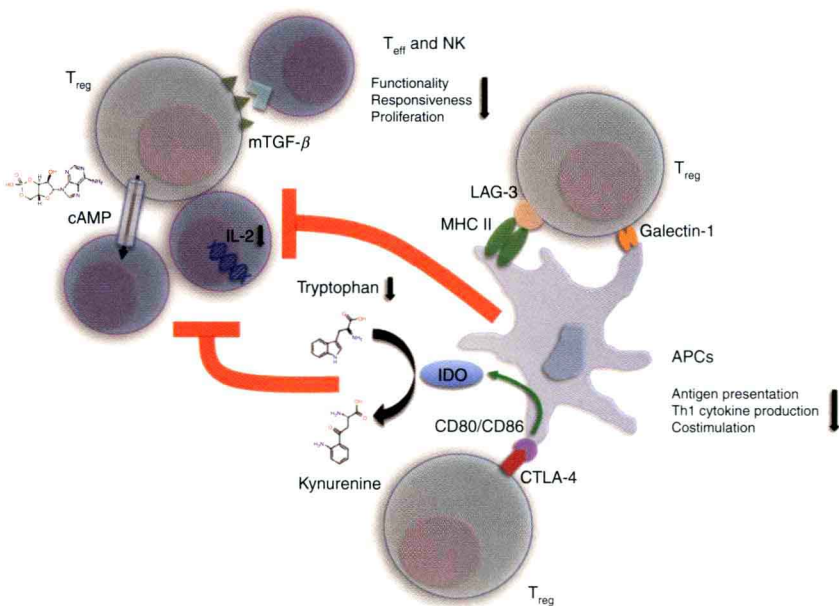
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A
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B
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C Consumption and redox phenomena

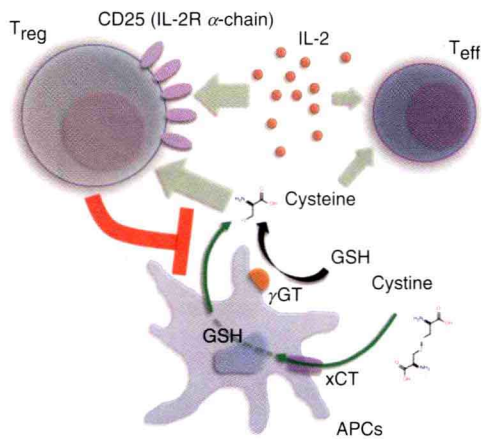


Fig. 1, Dimitrios Mougiakakos *et al.* (See Page 68 of this volume.)

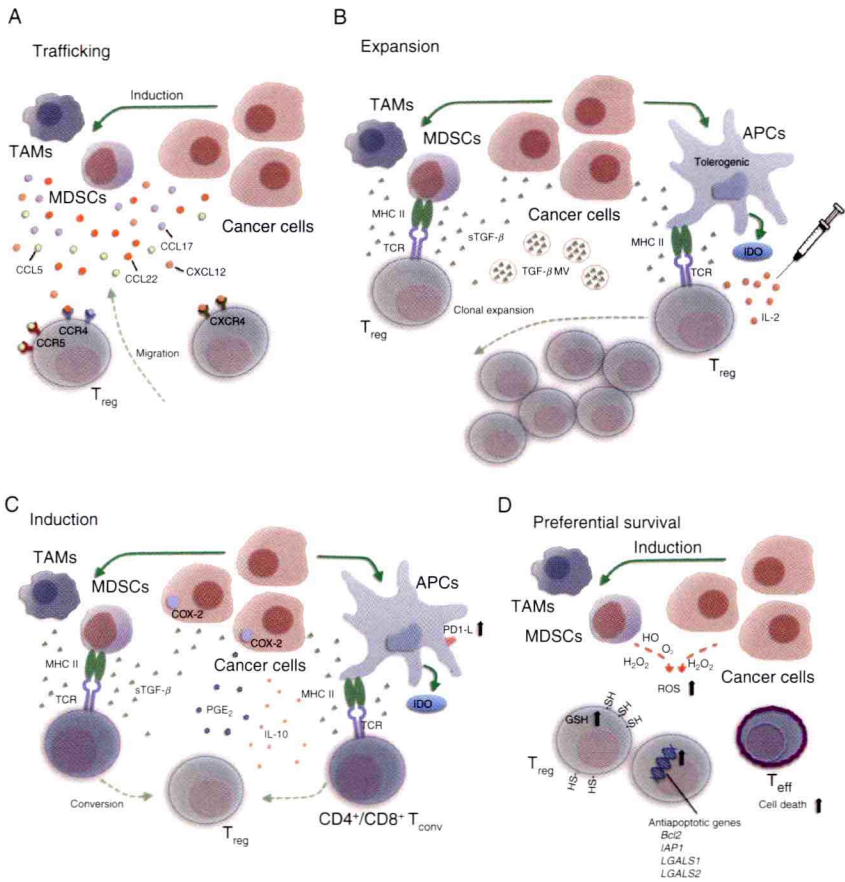


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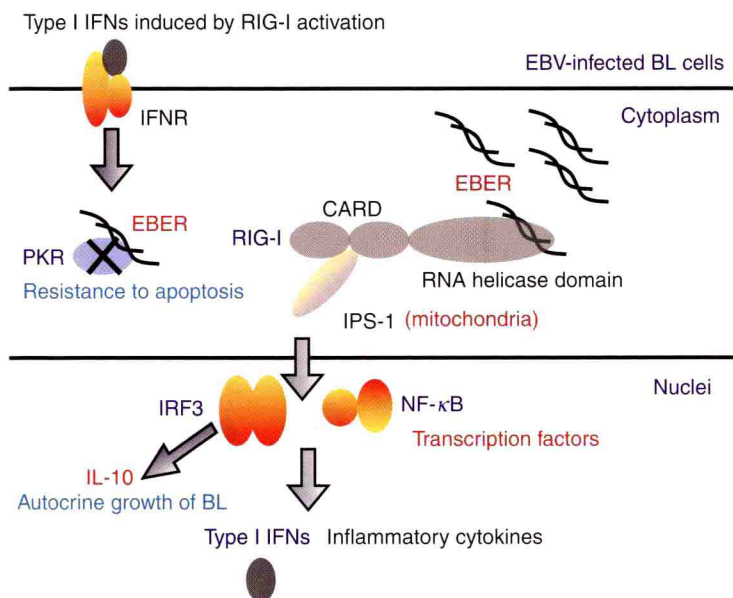


Fig. 3, Dai Iwakiri and Kenzo Takada (See Page 129 of this volume.)

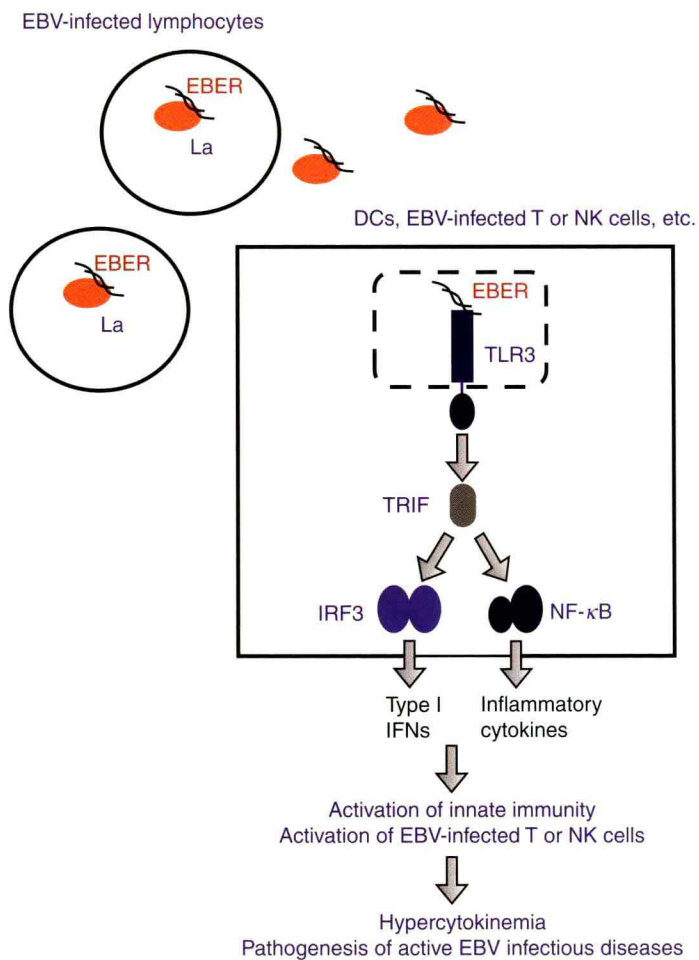


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Breaking Tolerance in a Mouse Model of Multiple Myeloma by Chemoimmunotherapy

Amir Sharabi and Nechama Haran Ghera

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A unique mouse model of multiple myeloma (MM), namely 5T2MM-bearing mouse, was useful for elucidating the pathophysiological mechanisms underlying the disease. Increased accumulation of suppressive CD4⁺CD25^{high}Foxp3⁺ regulatory T cells (Tregs) was observed in the thymus and lymphoid peripheral organs during disease progression. Adoptive transfer of Tregs, but not other thymocytes, from 5T2MM-bearing mice led to increased progression of disease manifestations in young syngeneic mice. Depletion of Tregs, a proposed strategy in cancer immunotherapy, was tested using cyclophosphamide (CYC), an alkylating agent with selective cytotoxicity. Both low- and high-dose CYC, administered to sick mice with hind limb paralysis, caused the paralysis to disappear, the plasma tumor cells in the bone marrow (BM) cavity to be replaced by normal cell populations, and the survival of the mice to be significantly prolonged. Low-dose CYC, which selectively depletes Tregs, decreased MM incidence, in contrast to high-dose CYC, which was generally cytotoxic, and did not reduce MM incidence. In contrast, low-dose CYC induced Tregs to become susceptible to apoptosis by down-regulating Bcl-xL and CTLA-4 in these cells, and by decreasing the production of IL-2 by

effector CD4 cells. This treatment consequently triggered the recovery of IFN- γ -producing natural killer T cells and the maturation of dendritic cells. Transient gradual depletion of Tregs in low-dose CYC-treated 5T2MM mice was maintained beyond 45 days. Thus, less frequent injections of low-dose CYC enabled us to recruit compatible immune-derived cells that would reduce tumor load and delay or prevent tumor recurrence, hence breaking immune tolerance toward MM tumor cells. © 2010 Elsevier Inc.

I. INTRODUCTION

Multiple myeloma (MM) is a progressive B-lineage neoplasia characterized by proliferation of clonal malignant plasma cells in the bone marrow (BM). The tumor cells secrete an immunoglobulin, usually monoclonal IgE or IgA in the serum and/or light chains in the urine. The progression of the disease may include anemia, lytic bone lesions, renal dysfunction, hypercalcemia, hypogammaglobulinemia, and peripheral neuropathy. Immune dysfunction is an important feature of the disease and leads to infections that are a major cause of morbidity and mortality. Moreover, it may promote tumor growth and resistance to chemotherapy. MM is characterized by numerous defects in the immune system including impaired lymphocyte functions, steroid-related immunosuppression, and neutropenia secondary to chemotherapy (Bergsagel and Kuehl, 2005). A reduced level of polyclonal immunoglobulins is a consistent feature of active MM, reflecting the suppression of CD19⁺ B lymphocytes that correlate inversely with the disease stage (Rawstron *et al.*, 1998). The relationship between myeloma plasma cells and the BM microenvironment is critical for maintaining the disease. Tumor cells and stromal cells interact via adhesion molecules and cytokine networks to simultaneously promote tumor cell survival, drug resistance, angiogenesis, and disordered bone metabolism. In addition, a number of immunologically active compounds are increased including transforming growth factor (TGF)- β , interleukin (IL)-10, IL-6, vascular endothelial growth factor (VEGF), Fas ligand, Mucin 1 (MUC-1), Cyclooxygenase (COX)-2, and related prostanoids and metalloproteinases (Pratt *et al.*, 2007).

Various drugs having immunomodulatory effects have been used in MM treatment. Thalidomide, shown to have potent anti-inflammatory, antiangiogenic, and immunomodulatory properties, was reported to have anti-MM activity as well (Bartlett *et al.*, 2004; Rajkumar *et al.*, 2002; Singhal *et al.*, 1999). Lenalidomine is another immunomodulatory drug used recently (Richardson *et al.*, 2006) in a NKT cell target combinatorial immunotherapy approach (Chang *et al.*, 2006).

Animal models mimicking human MM are useful for better understanding the pathophysiological mechanisms involved in the progression of the disease and for developing new therapeutic strategies. A series of murine

models were described by Radl *et al.* (1988), in which MM arose spontaneously in aging mice of the C57BL/KaLwRij strain with a frequency of 0.5%. A series of tumors have been propagated *in vivo* by intravenous transfer of the diseased BM into young syngeneic mice. This series of MM tumors represents the human form of the disease since their clinical characteristics involve selective localization in the BM, serum M component, angiogenesis, and adhesion and chemokine profiles that are similar to human myeloma (Asosingh *et al.*, 2000; Vanderkerken *et al.*, 1997). The BM microenvironment consists of extracellular matrix protein and BM stromal cells, osteoblasts, and osteoclasts that play a crucial role in the pathogenesis of MM cell growth and survival (Hideshima *et al.*, 2007).

T cell tolerance to tumor-associated antigens plays a significant role in immune evasion by tumors (Drake *et al.*, 2006; Zou, 2006). Naturally occurring and adaptive regulatory T cells (Tregs) are anergic cells with suppressive capabilities that constitute 5–10% of CD4 cells. These cells are induced early during tumor development and were shown to contribute to tumor tolerance (Peng *et al.*, 2002; Zhou and Levitsky, 2007). The mechanisms underlying these effects include inhibiting the activity of a variety of immune cells that are tumor specific such as effector CD4 cells, CD8 cells, dendritic cells (DCs), natural killer (NK) cells, natural killer T (NKT) cells, and B cells (Chen *et al.*, 2005; Ghiringhelli *et al.*, 2006; Lim *et al.*, 2005; Nishikawa *et al.*, 2005; Piccirillo and Shevach, 2001; Thornton and Shevach, 1998; Turk *et al.*, 2004). Phenotypically, these suppressor cells are characterized by their expression of certain surface and intracellular molecules, which include the following: the IL-2 receptor alpha chain (e.g., CD25), cytotoxic T lymphocyte-associated protein 4 (CTLA-4), and glucocorticoid-induced TNFR-related protein (GITR). Recently, the lack of CD127 expression was shown to predict functional Tregs in normal humans (Liu *et al.*, 2006), but it is relatively unstudied in tumor Tregs. The transcription factor Forkhead-box-p3 (Foxp3) is a more specific marker of Tregs (Hori *et al.*, 2003). Recently, it was demonstrated that Bcl-xL plays a role in the induction and suppressive function of Tregs, in addition to its antiapoptotic effect (Sharabi *et al.*, 2009).

The presence of Tregs in tumors is associated with a poor prognosis (Curiel *et al.*, 2004). Patients with many different types of cancers had increased numbers of Tregs in their blood, tumor mass, and draining lymph nodes. Increased numbers of Tregs in lung and ovarian cancers were first reported by Woo *et al.* (2001). Later it was demonstrated that high frequencies of Tregs are allocated not only at the proximity of tumors but also in peripheral blood, thus suggesting that an increased number of Tregs is a generalized phenomenon (Liyanage *et al.*, 2002). It is thought that active proliferation of Tregs rather than redistribution from other compartments is responsible for the tumor-associated increase in the numbers of Tregs (Wolf *et al.*, 2006).

Increased numbers of Tregs were found in patients with MM as well (Beyer and Schultze, 2006; Beyer *et al.*, 2006; Feyler *et al.*, 2009). Interestingly, *in vitro* expansion of Tregs could be induced in the presence of MM-specific antigens (Han *et al.*, 2008). The increased number of Tregs was associated with reduced immune effector functions (Han *et al.*, 2008), and was suggestive of the progression of malignant transformation (Beyer *et al.*, 2006).

Therapeutic approaches for breaking tolerance to tumor cells have been tried; the depletion of Tregs is the most studied strategy (Ercolini *et al.*, 2005; Ghiringhelli *et al.*, 2004; Shimizu *et al.*, 1999). Specific depletion of Tregs by anti-CD25 antibodies improved endogenous immune-mediated tumor rejection (Shimizu *et al.*, 1999) by enabling the development of tumor-specific CD8 cells and NK cells that reacted against tumors (Shimizu *et al.*, 1999). Nevertheless, despite the tumor antigen-specific immunity (Tanaka *et al.*, 2002), the tumors were not completely rejected (Jones *et al.*, 2002). Cyclophosphamide (CYC) was found to have specific effects on T cells, with tumor-inhibiting properties (Proietti *et al.*, 1998). This alkylating agent was shown to have beneficial effects in the treatment of MM, and to be associated with increased survival rates (Rivers *et al.*, 1963). It was reported that the beneficial effects of CYC were due to the removal of suppressor T cells rather than to the reduction in tumor burden (McCune *et al.*, 1998).

The use of various doses of CYC for depleting Tregs in different types of solid tumors has been reported. In this regard, low doses of CYC had a specific effect in depleting Tregs (Awwad and North, 1989; Ghiringhelli *et al.*, 2004). High-dose CYC also depleted Tregs but was less effective than the low-dose CYC in rejecting the tumor (Castano *et al.*, 2008). Thus, apparently the beneficial effects of low-dose CYC on tumor rejection may predominantly be immune mediated and less cytotoxic mediated. Indeed, the resulting depletion of Tregs by low-dose CYC augmented the immune response to cancer immunotherapy (Machiels *et al.*, 2001), unlike the high-dose CYC, which caused general immune cell depletion, and as a consequence, the concomitant depletion of CD4 cells and CD8 effector T cells that are required for developing an effective antitumor immunity (Castano *et al.*, 2008). Further, low-dose CYC inhibited angiogenesis and vasculogenesis (Kerbel and Kamen, 2004), and impeded tumor cell repopulation kinetics (Wu and Tannock, 2003). In agreement, mathematical analysis of the evolutionary dynamics of tumor populations predicted that the control of tumors by chemotherapy could be achieved using progressively lower doses and increasingly long intervals between doses (Gatenby *et al.*, 2009). Hence, it is suggested that a desirable effect of a chemotherapeutic compound would result in a tumor volume that is either stable or slowly increases for a prolonged period of time.