regulatory regions (about 1% of those surveyed), including a large number of hematopoietic transcription factor genes, but also several endothelial-specific genes, such as VE-cadherin. Thirty-six genes were in both datasets, that is, both direct targets of Sox17 and transcriptionally up-regulated.

It seems likely from the transcriptional profile and ChIP data that Sox17 is initiating a hematopoietic program while also maintaining the endothelial program, thus allowing expansion of a cell type stuck between 2 fates. This is distinct from the 2 previously studied regulators of endothelial hemogenesis: HoxA3, the negative regulator, promotes the endothelial program while preventing acquisition of the hematopoietic program; Runx1 on the other hand appears to erase the endothelial program while inducing the hematopoietic program.

It is interesting that several of the genes targeted and induced by Sox17 expression were actually screened in the initial experiment, and found to have no effect on proliferation of endothelial or pre-HPC cells. It may be that combinatorial effects explain the activity of Sox17. However, the genes in this initial panel were all selected for playing important or essential roles in early HPCs. Because hematopoietic differentiation occurs efficiently without intervention in this iPS culture system, pro-hematopoietic factors might not present a strong phenotype in this assay. On the other hand, anti-hematopoietic or pro-endothelial factors would be predicted to have clear phenotypes, the latter possibly explaining why Sox17 was found.

It is notable that in addition to a dual hematopoietic-endothelial phenotype, high levels of Sox17 expression drive the proliferation of this intermediate cell type. In theory at least, proliferation is not necessary for hemogenesis. Nakajima-Takagi et al show that under normal circumstances, high levels of Sox17 persist for only a short time, and in the earliest (endothelial) fraction, presumably at the moment of their hemogenic commitment. High levels of Sox17 thus appear to “kick-start” hemogenesis. Whether the concomitant proliferation might serve some biophysical purpose, facilitating bulging of cells out of the epithelial sheet for example, remains to be determined. In any event, the sustained proliferation of this transitional population by Sox17 is serendipitous in that it allows the generation of large numbers of these cells for study, which should facilitate a number of important experiments. For example, as the mechanism of hemogenesis is still very much a black box, it will be valuable to determine which of the 36 directly up-regulated targets are actually necessary for effects of Sox17 on differentiation and proliferation. It would also be interesting to study the cell biologic changes that occur as hemogenic endothelial cells convert into hematopoietic cells in a large scale culture synchronized en masse by Sox17 release. Finally, and most importantly, has Sox17 brought us any closer to the important goal of developing transplantable hematopoietic stem cells from human iPS cells in vitro? Having embryonic origins in a sustained hemogenic endothelium is a feature that distinguishes intra-embryonic definitive repopulating hematopoietic stem cells from their earlier yolk sac nonrepopulating cousins. It will therefore be very interesting to learn whether these Sox17-induced human iPS-derived hematopoietic progenitors have in vivo repopulating potential.

Conflict of interest disclosure: The author declares no competing financial interests.

●●●

LYMPHOID NEOPLASIA

Comment on Richter et al, page 423

Can NKT cells extinguish smoldering myeloma?

Don M. Benson Jr1

1THE OHIO STATE UNIVERSITY COMPREHENSIVE CANCER CENTER

One of the greatest challenges in all of medicine is to improve on the life of an asymptomatic patient; however, in this issue of Blood, Richter and colleagues share provocative new data taking steps toward accomplishing this goal.1 In this study, α-galactosylceramide (α-GalCer)–loaded dendritic cells (DC) were used in combination with lenalidomide to elicit multi-component, immune activation in patients with asymptomatic myeloma.1 Treatment appeared to be well tolerated and was associated with effects in several immune cell subsets. A reduction in M-protein was observed in 3 of 4 patients with measurable disease. This innovative approach builds nicely on prior studies and makes several important, new contributions to ongoing efforts toward effective immunotherapy of myeloma.

Multiple myeloma proceeds from a “pre-malignant” phase termed “monoclonal gammopathy of uncertain significance” (MGUS) through an asymptomatic “smoldering” phase to active, clinically symptomatic disease.2 While the course of MGUS is variable, most patients with asymptomatic myeloma will inexorably progress to manifest signs and symptoms requiring therapy and ultimately die of their disease. Although a topic of ongoing investigation, the standard of care for patients with asymptomatic myeloma remains vigilant observation and withholding therapy until signs or symptoms of active disease appear.2 Development of clinical myeloma from asymptomatic precursor states is associated with worsening immune dysfunction, generating increasing interest in the opportunity for early intervention.

REFERENCES


with immune-based therapies to prevent or delay this progression.2

When loaded into human DCs, α-GalCer, a potent ligand for Natural Killer T (NKT) cells, has been shown to activate and increase circulating invariant NKT cells in vivo.5-6 In a complementary manner, lenalidomide appears to induce favorable immunomodulation of T, natural killer (NK), and NKT cell subsets.5-7 Richter et al hypothesized that the combination of these therapies could lead to synergistic enhancement of immune activation against myeloma. After leukapheresis, monocytes isolated from patients with asymptomatic myeloma were cultured in granulocyte macrophage-colony stimulating factor and interleukin-4, and DCs were loaded with clinical grade α-GalCer (KRN7000; KHK). Patients received 3 cycles of low dose lenalidomide (10 mg by mouth days 1-21) and a fixed dose of 10 million DC (intravenously, day 8) on 28-day cycles. Extensive immune monitoring was performed, and clinical activity was determined 30 days after completion of all treatment.

Therapy was generally well tolerated and associated with several immunomodulatory events (see figure). Interestingly, whereas α-GalCer alone was previously shown to expand circulating NKT cells, an early and persistent decrease in NKT cells was observed in the present trial in combination with lenalidomide. NK cell expansion occurred, which appeared to be related primarily to lenalidomide, but increased expression of the activating receptor NKG2D on NK cells was also seen, particularly after infusion of α-GalCer–loaded DCs. CD16 expression on CD14(+) monoocytes and natural killer cell expression of NKG2D was also increased. Evidence to suggest SOX2-directed T-cell immunity was also observed.

Patients with asymptomatic myeloma received lenalidomide and autologous, α-GalCer–loaded DCs to stimulate NKT cells. While a reduction of NKT cells was observed in peripheral circulation, expansion of eosinophils and natural killer cells was observed. CD16 expression was increased on CD14(+) monocytes and NKT cell subsets.5-7 SOX2, an embryonal stem cell antigen previously identified as a possible immune target in plasma cell dyscrasias.8

The study by Richter and colleagues was small (n = 6, apparently because of lack of ongoing access to clinical grade α-GalCer) and raises several questions. First, it is unclear why α-GalCer alone led to NKT cell expansion in a prior study, but in combination with lenalidomide, NKT cells were decreased—perhaps this was related to trafficking of this subset out of peripheral circulation? Second, even though a low dose of lenalidomide (10 mg) was used, it is possible that this agent could have had a direct apoptotic effect on malignant plasma cells to account for the reduction of M-protein observed in some patients. Low dose lenalidomide may also modulate expression of NK cell ligands (eg, ULBP-1) to facilitate immune response against the disease.9 The study would have been strengthened with analyses of bone marrow plasma cells before and after therapy with α-GalCer and lenalidomide to detect any modulation of immune-cell ligand expression (such as CD1d and others) that may have contributed to immune-mediated rejection of myeloma tumor cells in a manner complementary to the immunomodulatory effects demonstrated. Third, the mechanism underlying the novel finding of eosinophil expansion in treated patients is also unclear. Finally, although requiring more extended follow up, it remains crucial to demonstrate that this approach would improve time-to-progression as a clinically relevant end point superior to M-protein reduction.

None-the-less, the study provides an important, first-in-human experience with α-GalCer and lenalidomide in myeloma. Lenalidomide has traditionally been paired with dexamethasone, a corticosteroid, which has formed the backbone of virtually every effective antmyeloma therapy to date. However, dexamethasone may attenuate lenalidomide’s favorable immunologic properties,10 and corticosteroids are associated with substantial risk of side effects and toxicities (including hypertension, glucose intolerance, osteoporosis, and psychologic effects) in an already vulnerable patient population. Thus, the development of novel antmyeloma therapies, which are steroid-sparing and augment lenalidomide’s immunomodulatory effects represents a compelling opportunity to advance the care of patients with myeloma particularly in the early intervention setting. Targeting NKT cells may also be particularly attractive in that this immune cell subset may serve to link innate and adaptive antitumor, immune resources.

Especially in attempts to improve the lives of asymptomatic patients, Hippocrates’ advice to “do no harm” remains paramount. Nonetheless, achieving prevention of (or substantially delaying) the development of the associated morbidity and mortality of active myeloma in patients with asymptomatic, “smoldering” disease would represent significant progress. The findings of Richter et al are certainly encouraging and warrant further inquiry into this approach.

Conflict-of-interest disclosure: The author declares no competing financial interests.

REFERENCES
2. Landgren O, Kyle RA, Rajkumar SV. From myeloma
Neutrophils are among the first innate immune cells to encounter invasive bacteria in host infection. The classical mechanism by which neutrophils kill bacteria is through phagocytosis and digestion of pathogenic microbes. Microbes are then eradicated by the release of reactive oxygen species and cytoxic granule proteins inside the resulting phagosome. For many years this was considered the principal mechanism by which neutrophils controlled the growth of pathogenic bacteria.

Recent reports suggest that neutrophils have a wider repertoire of weapons against bacteria than previously appreciated. Neutrophils have been demonstrated to arrest the growth of pathogenic bacteria by the production of extracellular traps (NETs) resulting from the release of nuclear DNA together with antimicrobial proteins. The discovery of NETs has led to the notion that neutrophils are able to exert control over extracellular bacterial growth in addition to intracellular killing of bacteria. This has led to a paradigm shift in the role of neutrophils maintaining immunity.

In this issue of Blood, Timár and colleagues provide fascinating evidence for another immunologic mechanism in neutrophils that is directed at controlling the growth of bacteria. These are extracellular microvesicles, also known as microparticles or ectosomes, released by neutrophils in response to opsonized Staphylococcus aureus or Escherichia coli. Neutrophils are known to generate microvesicles spontaneously and in response to triggers such as f-Met-Leu-Phe, tumor necrosis factor, lipopolysaccharides, opsonized yeast and bacteria, and others. Previous reports have shown that neutrophil microvesicles affect platelets, endothelial cells, and macrophages. The report by Timár et al shows for the first time that neutrophil microvesicles also possess antibacterial activity. But their antibacterial activity depends on what triggers their formation.

In the paper by Timár and colleagues, microvesicles from human peripheral blood neutrophils were segregated into 3 different categories based on the activation status of the neutrophils from which they were derived: s-MV (for spontaneous), p-MV (for phorbol ester–stimulated), and b-MV (for bacterially induced microvesicles). Analysis of the protein content, size, and morphology of MV revealed that these were present in 2 sizes (~100 and ~500 nm, most of which were the larger size).
Can NKT cells extinguish smoldering myeloma?

Don M. Benson Jr