IL-36 proteins do not easily suggest a consensus cleavage site. The logjam was broken when Towne and colleagues showed that all 3 IL-36 agonists and IL-36Ra need to be processed at one particular position (apparently zero tolerance), close to the N-terminus of the precursor protein. Once supplied to cells that have IL-36 receptors in this “polished” form, the vast functional discrepancy disappears between IL-36 and IL-1. What we still do not know is which proteases in nature can “polish” IL-36. Knowing this will probably reveal much about the biologic function of IL-36.

In their paper, Vigne et al argue that responses to IL-1–like cytokines have more to do with the cell types that express them than to intrinsic differences surrounding the receptors; a very sensitive position given that the receptors themselves are so similar and that IL-1Rac1 is reused for IL-1, IL-33, and IL-36. They show that both dendritic cells and CD4+ T lymphocytes from mice have IL-36 receptors and that both cell types respond to “polished” IL-36. Dendritic cells responded more strongly to IL-36 than to IL-1 and the response was a barrage of cytokines and the up-regulation of cell-activation and antigen-presentation markers, suggesting that IL-36 should be better than IL-1 at promoting antigen presentation. In other experiments, Vigne and colleagues showed that IL-36 functions as an adjuvant in vivo.

There are differences between the response to IL-36 and IL-1 in CD4+ T cells too. It is not IL-1–like that IL-36 strongly induces interferon-γ and induces IL-4 significantly. These are activities that would be expected from IL-18 and IL-33, respectively. In isolation too, in these authors’ hands, IL-1 induced IL-17 production strongly while IL-36 did not. Vigne and colleagues come to the conclusion that stimulation by IL-36 might favor the differentiation of the TH1 subset of T-helper cells rather than the TH17 subset, which are heavily implicated in autoimmune diseases. IL-1, by contrast, is essential for the TH17 response in vivo and drives the production of substantial amounts of the highly pro-inflammatory cytokine IL-17 from cultured spleen T cells. However, the story might not end this simply because strong IL-17 production in this type of assay in response to IL-1 usually requires IL-23, and Vigne et al have not yet reported the effect of co-stimulation of T cells with IL-36 and IL-23.

Complementing the work in the current paper, there is now strong genetic evidence that proper regulation of IL-36 in the skin is vital in humans. Individuals who have no functional IL-36Ra gene develop life-threatening episodic pustular inflammation; a clear case of immune dysregulation in the skin as well as more generalized episodic fever.

Now that we have the reagents, there will be plenty to investigate about the IL-36 system. We can now do so in the faith that it is actually functional!

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

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Immunodeficiency diagnosis: a Mondrian or Pollock scenario?

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In this issue of Blood, Zhang et al report that hypomorphic mutations in FHL-related cytokines in FHL-causing genes correlate with a later-onset and a more indolent course in adult patients. Another important study on the same disease led to the identification of alterations in an evolutionarily conserved intronic region, thus highlighting the importance of searching for aberrations outside the coding region of the implicated gene.

Familial HLH is well recognized in children but rarely diagnosed in adults. Moreover, even though alterations of at least 7 genes are associated with the disease, in the majority of patients the molecular defect cannot be found.

The huge number of congenital disorders of the immune system discovered in the past 20 years has greatly contributed to the understanding of the physiology of the immune response. Remarkably, the easy availability of the “sick cell” gave the unique opportunity to search for an intimate link among clinical phenotype, genetic alteration, and the pathogenic mechanism of that individual immunologic functional abnormality. The discovery of genetic alterations in so many molecules led to an unexpected dissection of the numerous functional and biochemical pathways into distinct molecular events, thus favoring a detailed understanding of the role of that specific molecule. What important information came out of the discovery of genetic alterations of the common γ chain besides its pathogenic role in the SCID-X1? Gamma chain and T-cell development, the role of several γ-related cytokines in T-cell differentiation and functions, molecular therapy and immune reconstitution through gene transduction, and the general role of the molecule in cell-cycle progression and tumorigenesis are only a few examples that highlight the importance of studies of primary immunodeficiencies (PIDs) that go far beyond the understanding of the cause of a disease. Very often, studies in this field highlight shared aspects, which also help unravel
complex issues in other fields of modern medicine.

It is not surprising that the greater the number of the distinct immunologic disorders, the greater the diversity of presenting signs. Much time elapsed from the paradigm that a severe PID should have been ruled out if the patient had recovered from an infection by the time expected. Thus, no one would have imagined 10 years ago that the onset of a severe PID could be during adulthood. Actually, an eminent physician scientist in the field, Jean-Laurent Casanova, in a lecture at the European Society of Immunodeficiencies, argued that every time the clinical course of an infection is life threatening there is an underlying immunodeficiency to discover. Although this opinion may be, to some extent, extreme, if it will be proven true in the future, it certainly implies that the true prevalence of congenital immunodeficiencies will not be defined until all patients with very severe infectious diseases have been investigated for an underlying immunologic disorder.

The interesting study by Zhang et al here from the Filipovich group reports that hypomorphic mutations in FHL-causing genes correlate with the later-onset disease, as previously reported in this journal in one family. This genetic alteration is associated with a more indolent course, resulting in adult presentation with a few cases even in their seventies. This study also points out that it is quite difficult to define a boundary between causative genetic alterations and a genetic variation that predisposes to the disease. In this study, the A91V-PRF1 genotype was found in half of adult patients with FHL in both the heterozygous and homozygous states. This variation has previously been found in the general population, with its prevalence ranging between 4% and 7%. However, the prevalence of the variation in FHL patients is much higher, up to 26% in a cohort of well-characterized patients. Taken together these data imply that this variation is an important genetic susceptibility factor. A number of studies also document that the A91V substitution is not clinically neutral, even though it may variably affect cytotoxic activity. Accordingly, it is not surprising that the greater the diversity of presenting signs, the more complex the diagnosis becomes. At the very beginning, when it became clear that most of the defects of cytotoxic activities were related to alterations of molecules implicated in the cytolytic signaling pathway (see figure panel A), physician-scientists thought of the disease pathogenesis in too simplistic a way. At least 7 genes (PRF1, MUNC13-4, STX11, RAB27A, STXBP2, SH2D1A, and BIRC4) are known to be associated with FHL, but unfortunately, in the majority of patients with a well-established clinical and functional diagnosis of HLH, molecular diagnosis is still not possible. Paralleling contemporary art masterpieces, many of us thought at the very beginning of Mondrian linearity (figure panel B). Indeed, the complexity of the relationship among clinical phenotypes, altered biochemical pathways, and individual gene alteration is rather comparable with Jackson Pollock complexity (figure panel C), which seems to clearly depict the cross-interference between distinct pathways, the huge number of molecules in that specific pathway, the number of distinct genetic and environmental predisposing factors, and the functional role of distinct molecular alterations of that molecule. It should also be kept in mind that many viruses may persist or alter cell physiology in the absence of any genetic disorder. Another study in this issue of Blood by Meeths et al on the same disease highlights the importance of also searching for aberrations outside the coding region of UNE13D gene in patients with FHL. This study addresses the important issue of defining a pathogenic mechanism in FLH patients with no molecular diagnosis. The alteration was located in an evolutionarily conserved intronic region and was consistent with a loss-of-function mutation in that it resulted in defective natural killer cell degranulation and cytotoxicity, as well as absent MUNC13-4 expression. The conclusion reached by these authors is that the search for alterations outside the coding regions helps in the diagnosis as well as in deciding on therapy for such patients. Furthermore, the strategy of sequencing of evolutionarily conserved noncoding regions should be applied to the diagnostics of the whole field of primary immunodeficiencies. In a number of cases of PIDs, despite a clear clinical and functional phenotype suggestive of a specific diagnosis, no molecular confirmation can be reached leading to the hypothesis that a novel altered gene might be responsible. Although this may be true in many cases, the possibility of a genetic alteration of a noncoding region should be taken in consideration.

A further implication of these observations is that the dream of screening through microarray technologies all the genes coding for molecules in a function-related pathway is likely to remain a dream because of the importance of the noncoding regions. A further
point of interest in the study by Meeths et al is the observation of a 9-fold reduction in the transcription of the mutated UNC13D allele relative to the wild-type in lymphocytes. The relative frequency of the transcript from the mutated allele in CD4+, CD8+, and CD56+ cells was significantly less than that observed in CD14+ cells, suggesting a role for the intronic mutation in cell-specific transcription of the UNC13D gene. One could argue that in the future the search for protein expression or gene transcription has to be performed only in the presumed sick cell to be informative. In light of these considerations, going back to the functional assessment as the key point of the diagnostic procedure for PIDs might prove to be the more appropriate strategy.

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Adipokines in MM: time to trim the fat

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In this issue of Blood, Fowler et al provide new evidence that the adipokine adiponectin may be a therapeutic target in myeloma and myeloma-associated bone disease.1 The global epidemic of obesity has been linked to several diseases such as metabolic syndrome, atherosclerosis, and several cancers including myeloma (MM).2 Several studies have now clearly shown that the accumulating fat does not simply serve as an inert storage site, but as a dynamic endocrine organ secreting hormones called adipokines.3 Adipokines play a key role in regulating energy homeostasis as well as inflammation. Adiponectin was initially identified in mid-1990s as an adipocyte-derived protein similar to complement 1q, and later shown to have anti-inflammatory properties.3,4 Several adipokines, particularly leptin and adiponectin, have also been implicated in regulating the risk of developing cancers.2

The studies by Fowler et al now add myeloma to the list of these cancers. The initial identification of adiponectin as a potential target in myeloma was based on gene array analysis of the marrow microenvironment between KaLwRij mice that permit growth of murine 51M myeloma cells, versus nonpermissive but closely related G77B6 mice. Adiponectin was one of several genes in this analysis and the role of other differentially expressed genes may well be equally relevant and deserve further study. Nonetheless, increased tumor burden and MM bone disease were clearly observed in adiponectin-deficient mice and pharmacologic induction of adiponectin using an apolipoprotein peptide mimetic L-4F led to the reduction of tumor growth and prevention of MM bone disease in this model. L-4F can in principle also impact other targets, but the antitymoma effects of L-4F seem to require adiponectin as they were lacking in adiponectin-deficient mice. These provocative findings suggest the need for further study to better understand the mechanism(s) by which host adiponectin expression might regulate MM growth. Adiponectin seems to have direct effect on both MM cells and bone cells. However, the effects of adiponectin in promoting an MM-permissive microenvironment may be multifactorial and additionally include effects on the recruitment of innate immune cells, such as macrophages.5 These elegant studies suggest a novel concept that the expression of adipokines such as adiponectin in the tumor microenvironment may regulate the permissive state of the marrow microenvironment to MM growth.

What are the potential clinical implications of this work? Detection of low adiponectin levels may suggest an increased risk for MM.6,7 While adiponectin levels are inversely related to obesity (a known risk factor for MM),8,9 the authors were careful in their use of controls matched for body mass index. Patients with monoclonal gammopathy of undetermined significance (MGUS) who progressed to MM in this small sample had lower levels of serum adiponectin compared with those that did not progress. However, this risk was restricted largely to females and the reasons behind this apparent sex bias are not readily evident at present. Nonetheless, further systematic investigation of this biomarker in ongoing prospective studies of monoclonal gammopathies may yield further insight.

The concept that the permissive state of tumor microenvironment for MM growth may be altered by adipokines such as adiponectin has important implications for preventing clinical MM. While the outlook for clinical MM has improved considerably in the past decade, the net gains in improving mortality have been much more modest compared with tumors, wherein early detection and prevention have instead been emphasized.10 The current studies set the stage for future investigations targeting obesity or pharmacologic manipulation of adipokines (such as adiponectin) as an attractive strategy for the prevention of clinical MM in selected cohorts. It is certainly now time to trim the fat.
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