IL-36 proteins do not easily suggest a consen-
sus 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 pre-
cursor 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 re-
sponses 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 sensible position given that the
receptors themselves are so similar and that
IL-1RaCp 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 ante-
gen presentation. In other experiments, Vigne
and colleagues showed that IL-36 functions as
an adjuvant in vivo.

There are differences between the re-
sponse 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 iso-
lated too, in these authors’ hands, IL-1 induced
IL-17 production strongly while IL-36 did not.
Vigne and colleagues come to the conclu-
sion 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 produc-
tion 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 sys-
tem. We can now do so in the faith that it is
actually functional!

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Immunodeficiency diagnosis: a Mondrian
or Pollock scenario?

Claudio Pignata and Giuliana Giardino FEDERICO II UNIVERSITY

In this issue of Blood, Zhang et al report that hypomorphic mutations 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 chil-
dren 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 can-
not be found.

The huge number of congenital disorders
of the immune system discovered in the past
20 years has greatly contributed to the under-
standing of the physiology of the immune re-
sponse. Remarkably, the easy availability of the “sick cell” gave the unique opportunity to
search for an intimate link among clinical phe-
totype, genetic alteration, and the pathogenic
mechanism of that individual immunologic
functional abnormality. The discovery of ge-
etic alterations in so many molecules led to an
unexpected dissection of the numerous func-
tional and biochemical pathways into distinct
molecular events, thus favoring a detailed un-
derstanding of the role of that specific mole-
cule. What important information came out of
the discovery of genetic alterations of the com-
mune γ chain besides its pathogenic role in the
SCID-X1? Gamma chain and T-cell develop-
ment, the role of several γ-related cytokines in
T-cell differentiation and functions, molecu-
lar 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

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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.

Unfortunately, the more complete the understanding of the disease pathogenesis, 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 cytotolytic 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, STXB2, 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 per se 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 alterations outside the coding region of UNC13D 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 CD4+ 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

Madhav V. Dhodapkar YALE UNIVERSITY

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 51γ myeloma cells, versus nonpermissive but closely related C57Bl6 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 anticytoma 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 narrow 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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