cytokines, such as IL-2, IL-4, and interferon-\(\gamma\) (IFN-\(\gamma\)), with primary (keyhole limpet hemocyanin [KLH]) and recall (purified protein derivative [PPD]) antigens and the mitogen concanavalin A (ConA). However, in previous studies the lymphocytes were found to produce these cytokines when stimulated with phytohemagglutinin (PHA) or with phorbol ester (PMA)–ionomycin. Specifically, when the CD26\(^+\) CD4 cells immediately surrounding the Reed-Sternberg cells were purified and stimulated with PMA ionomycin, they produced IL-4 and IFN-\(\gamma\). The potential to produce IL-4 was the reason why these cells were previously considered Th2-like.\(^2\) The absence of IL-2 production upon stimulation is also associated with the anergy. The exact nomenclature of these cells is thus a matter of semantics. In addition to the IL-10–producing cells (Tr1), there are also transforming growth factor \(\beta\) (TGF-\(\beta\))–producing cells present in the infiltrate, and these have been termed Th3.

The findings by Marshall and colleagues indicate that there are variations in the populations involved in different cases. It can be concluded that, as an overall population, the infiltrating lymphocytes do not have Th1 type functions and are probably attracted into the tissues by chemokines TARC and MDC as CCR4-expressing Th2 cells. Although these cells do not spontaneously produce IL-2 or IL-4, they produce IL-10, despite not being fully activated, and therefore function as Tr1 cells.

The major remaining question is what causes the predominance of T cells with suppressor activity in Hodgkin lymphoma. It appears that Reed-Sternberg cells, although they have the genotype of B cells, execute a functional program that is similar to antigen-presenting cells but results in tolerance. Mechanisms include the production of immunosuppressive cytokines like IL-10, especially in Epstein-Barr virus–positive cases, and TGF-\(\beta\), especially in nodular sclerosis cases, as well as the expression of FAS ligand that induces cell death in FAS-expressing activated T cells, while the Reed-Sternberg cells themselves are protected by overexpression of cFLIP (Fas-associating protein with death domain–like IL-1\(\beta\)–converting enzyme [FLICE]–inhibitory protein) or infrequently by FAS mutation.\(^3\)

The relevance of these findings is that they may allow a better design of new treatment modalities. There are indications that the infiltrating cells in fact support the growth and survival of the Reed-Sternberg cells, and therefore blocking chemokines to prevent the influx of T cells may be effective. On the other hand, blocking of the immunosuppressive signals, such as IL-10 and TGF-\(\beta\) cytokines, or the removal of the suppressor regulatory T cells may enhance the effect of adoptive transfer of cytotoxic T cells.\(^4\)

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More complexity in MLL-associated leukemias

The mixed lineage leukemia gene (\(\text{MLL, also known as } \text{ALL-1, and } \text{HRX}\)) has rightly attracted much interest as a major player in leukemia.\(^1\) \(\text{MLL’s central role is clear by its involvement with over 30 different partner genes in recurrent translocations. As if this were not enough, } \text{MLL is also implicated in leukemia by overexpression in the absence of overt mutations or by acquisition of partial tandem } \text{MLL duplicates. What, then, accounts for the leukemogenicity of } \text{MLL? Might there be some common functional thread tying together many of the fusion genes and } \text{MLL overexpression? At least one strong clue has emerged from the recognition that a major function of } \text{MLL, like its Drosophila homolog Trithorax, is to serve as a maintenance factor for the expression of many members of the Hox family of transcription factors. } \text{Hox genes are now recognized as major components of the regulatory machinery of primitive hematopoietic cells. Strikingly, multiple lines of evidence link } \text{Hox genes directly to leukemic transformation.}\(^2\,3\) This evidence includes induction of leukemia in mice following engineered overexpression of certain \(\text{Hox genes (eg, } \text{HOXA9 and } \text{HOXA10} \text{) and the observed overexpression of multiple } \text{Hox genes in human leukemia and, notably, in } \text{MLL-associated leukemias. Perhaps most convincingly, multiple members of the Hox family, } \text{HOXA9 for one, have been identified as translocation partners in leukemias with the common partner } \text{Nucleoporin 98.}\)\(^4\) Thus, a satisfying model for some if not all \(\text{MLL-induced leukemias would be through induced deregulation of key } \text{Hox target genes. Strong support for this has recently been reported by Cleary and colleagues, who found that } \text{MLL-ENL lost leukemogenicity in bone marrow cells taken from } \text{Hoxa7 or } \text{Hoxa9 knockout mice.}\)\(^5\) The jump to a unifying model involving \(\text{MLL and } \text{HOXA9}, \text{however, is not without a tumble or two as indicated in the article by Kumar and colleagues (page 1823) in this issue of Blood. Their studies reveal unubated leukemogenicity by the fusion gene } \text{MLL-AF9 in the absence of } \text{Hoxa9. While there were clear influences of } \text{Hoxa9 on the phenotype of the leukemia, the essential transformation was not altered by the presence or absence of } \text{Hoxa9. The striking differences between these 2 recent studies involving related partner genes may be a consequence of several experimental and biologic differences. Kumar and colleagues have used a gene knock-in model of } \text{MLL-AF9 fusion rather than retroviral overexpression; the fusion genes may indeed have differential effects on } \text{Hox targets, rendering other members of the cluster more or less important. Indeed, multiple members of the Hox A cluster were observed to be up-regulated by } \text{MLL-AF9, making it possible that additive levels of } \text{Hox gene expression may be} \)
critical for hematopoietic effects. These studies caution against oversimplification in thinking about the likely complex paths from MLL to leukemia but further highlight the potential linkage to Hox genes and the power of genetic mouse models for investigating human disease.

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HIV envelope becomes unhinged by PDI for entry

Almost 10 years ago, Ryser et al1 reported that cleavage of 2 disulfide bonds in the gp120 surface component of the HIV-1 envelope is required for virion entry into CD4-bearing cells and that cell surface protein disulfide isomerase (PDI) is responsible for this effect. Since that initial report, there have been numerous structural and mechanistic studies of envelope binding, yet exceedingly few of these studies have incorporated any model of dynamic changes in gp120 disulfide bonding. The notable exceptions are papers from Fenouillet et al2 and Barbouche et al3 that have confirmed the original Ryser et al findings. Now, in this issue of Blood, Markovic and colleagues (page 1586) weigh in as the third group to confirm these findings, and they provide new information about the location of PDI in previously undescribed domains of the cell membrane.

The basic information is as follows: HIV virions, which bear perhaps only a dozen or so envelope spikes, use gp120 to bind to CD4, but this alone serves only to attach the virion loosely to the cell surface. Biophysical evaluation indicates that gp120 has a relatively short off-rate from monomeric CD4, although binding by multiple spikes could increase the avidity of binding.4 An alternative explanation is that once gp120 detaches, it has a high probability of reattaching to an adjacent CD4 molecule on the cell surface, producing the appearance of a long off-rate through a newly recognized phenomenon termed “allovalency.”5 This explains the long-puzzling finding that soluble CD4 or anti-CD4 antibodies can prevent HIV infection even if added a few minutes after virion adsorption. Eventually, however, gp120 moves laterally along the membrane surface until it collides with a patch of PDI in a domain of the membrane that Markovic et al distinguish from a typical lipid raft. There, PDI reduces 2 disulfide bonds in gp120, producing conformation changes that likely stabilize the binding of gp120 to CD4 and expose the V3 loop for subsequent binding to the chemokine coreceptor. Following this, gp41 undergoes rearrangement into its fusigenic intermediates and entry occurs (Markovic and colleagues and Reysen et al, Fenouillet et al, Barbouche et al, and Gallina et al).

Taken in aggregate, the reports from these 3 groups have profound implications for our understanding of the HIV virion surface. As shown by Kwong et al, the relative invariant CD4 binding site in gp120 is kept recessed to prevent access by otherwise neutralizing antibodies. The initial CD4 binding event is but a prelude to PDI-mediated reduction that unfolds the envelope into a more open conformation that presumably binds more favorably to CD4 and to the chemokine coreceptor. If evasion of neutralizing antibodies is the likely evolutionary reason for this shuffling two-step at entry, then it would be interesting to know if a PDI-rearranged envelope is a better vaccine immunogen for raising neutralizing antibodies. PDI is too ubiquitous an enzyme to use as a drug target.6 However, just as drugs have been developed that block CD4 binding, chemokine coreceptor binding, and now, most successfully, gp41-mediated fusion, it will be interesting to learn if the PDI-rearrangement step in gp120 could provide a new therapeutic target.

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Monoclonal antibodies in the treatment of chronic lymphocytic leukemia: if only it were simple

The necessary condition to cure any malignancy is to achieve a complete response (CR), which for years has been extremely rare in chronic lymphocytic leukemia (CLL). Fortunately, the days in which treatment of CLL revolved around the use of
More complexity in MLL-associated leukemias

Keith Humphries

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