proliferation and mutation, initiating another cycle of selection and differentiation. In this cyclic re-entry model, the molecular machinery driving V gene mutation—now known to be the enzyme activation-induced cytidine deaminase (AICD)—was associated intimately with B cell division and thus separated from the processes associated with differentiation, processes that are entirely dependent on the activity of the GC resident, CD4+ helper T cells, now known as follicular helper T (Thf) cells. An expectation of this model, in addition to that of the B-cell movement between zones, was that there would be molecular differences between the 2 populations of GC B cells, reflecting their different activities.

The mechanics underpinning GC function remained uncertain until the application of multiphoton microscopy to these structures during immune responses in mice (reviewed in Victora and Nussenzweig). Victora and colleagues used a photo-activatable form of the fluorescent protein GFP to uniquely label and thus isolate DZ and LZ B cells based on their in vivo location. Once isolated by flow cytometry, these 2 cell types were interrogated by molecular methods to reveal unique attributes, including the genes up-regulated uniquely in each zone and thus providing molecular signatures for each population. These signatures, not surprisingly, included cell-surface proteins that facilitated the routine isolation and characterization of these B cells. Chief among these markers were CXCR4, already identified as crucial for the correct function of GCs in mice, CD83 and CD86. Thus in mice, GC DZ B cells are CXCR4hi, CD86lo, and CD83lo while LZ B cells are the inverse, CXCR4lo, CD83hi, CD86hi. This identification allowed detailed characterization of these cells, which validated many aspects of the original model with DZ cells being significantly enriched for proliferation and LZ B cells interacting with GC T cells, driving the force behind selection and differentiation.

Now, Victora and colleagues have applied the mouse fractionation scheme to human GC B cells and found, more or less, complete concordance. Human tonsil GC B cells can be partitioned by CXCR4 and CD83 (CD86 is not useful), the populations thus identified correspond to the expected histologic loci, proliferation as measured by DNA content occurs predominantly in the DZ and, in addition, there is significantly increased expression of AICD in DZ B cells. Victora et al clarify other attributes of these 2 B-cell types. For example they note no major difference in cell size or granularity but increased expression of CD27—a marker normally associated with memory—on DZ B cells. Now able to identify and purify these 2 types of human GC B cells, Victora et al analyzed gene expression. They identified a modest number of differences between DZ and LZ B cells and, perhaps not surprisingly, found that the majority of the differences between the human B-cell subsets were also different between the equivalent mouse GC B-cell subsets. Thus, they defined through this cross-species comparison, gene-expression signatures that identify robustly and across species, DZ and LZ B cells. In fact, the authors make the point that the DZ and LZ B cell gene expression patterns are so similar that they should be considered stages of activation within 1 population rather than 2 discrete populations.

The final aspect of the work reported by Victora and colleagues was the examination of human GC-derived B-cell lymphomas for the DZ and LZ gene-expression signatures. The authors examined a series of B-cell non-Hodgkin lymphomas, comprising several distinct lymphoma subtypes, and found all types except 1 were assigned reproducibly and with great confidence to the LZ expression group. This included diffuse large B-cell lymphomas (DLBCL) of activated B cell (ABC) and GC types, follicular lymphoma and many cases of Burkitt lymphoma (BL). The remaining fraction of BL, however, segregated reasonably closely with the DZ expression signature. Closer examination of the BL cases revealed their DZ and LZ partition corresponded very closely with a previous division of these lymphomas into molecular and nonmolecular BL. It is concluded that the tumors resemble the LZ expression profile predominantly because of their expression of CD40/LMP1 and related signaling response genes, while loss of these signatures confers the DZ match.

In summary, there now is a clear manner to recover GC B cells from 2 compartments that are associated with the key functions of the GC: mutation, proliferation, selection, and differentiation. This will surely lead to greater insights into the initiation, propagation, and cessation of these crucial processes in both healthy and disease states. Similarly, combining knowledge of the migration route of GC B cells and the ability to recover the cells at the beginning and end of the track will also lead to better understanding of the complex but essential process of cell movement. The association of the zonal expression signatures with particular B-cell lymphomas may also lead to further insight into the origin or occurrence of the transformative events that collectively constitute lymphoma development.

Conflict-of-interest disclosure: The author declares no conflict of interest.

REFERENCES
Gamma delta (γδ) T cells expressing the Vγ9Vδ2 T-cell receptor account for approximately 5% of peripheral blood T cells. Many solid tumor and leukemia/lymphoma cells are susceptible to Vγ9Vδ2 T cell–mediated killing.2-4 Antitumor reactivity and the ability to intentionally increase this activity (see below) have raised great interest in exploring the immunotherapeutic potential of Vγ9Vδ2 T cells. In contrast to conventional αβ T cells, γδ T cells do not recognize antigenic peptides presented by MHC class I or class II molecules. It has been shown that human (and primate) γδ T cells expressing the Vγ9Vδ2 T-cell receptor recognize phosphorylated nonpeptide metabolites that can be secreted by many microbes. Such microbial “phosphoantigens” are extremely potent and specific activators of Vγ9Vδ2 T cells and cause their transient increase in the peripheral blood during the acute phase of many bacterial and parasitic infections.6 The microbial phosphoantigens are intermediates of the prokaryotic nonmevalonate pathway of cholesterol synthesis, and are active at pico- to nanomolar concentrations. Eukaryotic cells use the mevalonate pathway for cholesterol synthesis. Isopentenyl pyrophosphate (IPP), a naturally occurring endogenous phosphoantigen, is also seen by the Vγ9Vδ2 T-cell receptor but requires 3-log higher (micromolar) concentrations for γδ T-cell activation. Such concentrations do not accumulate in normal cells. On cellular transformation, however, much higher concentrations of IPP are produced, which then can be sensed by γδ T cells as tumor-associated antigen.

Interestingly, the levels of IPP production can be easily manipulated. Aminobisphosphonates (n-BP), which are in clinical use for the treatment of osteoporosis and bone metastasis in some cancer patients, inhibit an enzyme that degrades IPP, whereas statins reduce the endogenous production of IPP.1 In consequence, treatment of tumor cells with n-BP increases their susceptibility to γδ T cell–mediated lysis, because of enhanced IPP production.2,3 As a matter of fact, the therapeutic application of n-BP together with low-dose IL-2 results in increase of peripheral blood γδ T-cell numbers associated with some clinical benefit in patients with lymphoid malignancies7 or solid tumors.8

T-cell receptor gene transfer and mutagenesis studies have established that the T-cell receptor is crucially involved in the activation of Vγ9Vδ2 T cells by microbial or tumor-derived phosphoantigens. However, the mechanism of phosphoantigen recognition has remained undefined. Early studies indicated that such phosphoantigens do not require antigen processing nor do they require presentation by classic MHC molecules or MHC-related molecules such as CD1. Interestingly, however, a level of species restriction was noticed in that human but not murine cells were found capable of presenting such phosphoantigens to human γδ T cells. Previous work suggested that an ectopically surface-expressed ATPase might be involved in the cell-surface presentation of phosphoantigens.9 The work now reported by Harly et al represents a significant advancement in our understanding of how phosphoantigens activate human Vγ9Vδ2 T cells.1 These authors describe a central role for CD277, a member of the butyrophilin molecules, in this process. Butyrophilins are distantly related to costimulatory molecules of the B7

Role of CD277/butyrophilin-3A in triggering human γδ T-cell activation. (A) The ubiquitously expressed CD277 molecule is not recognized by the Vγ9Vδ2 T-cell receptor (left). Upon incubation with agonistic anti-CD277 mAbs, changes (mobility and/or conformation) are induced in the CD277 molecule such that Vγ9Vδ2 T cells are activated in a T-cell receptor–dependent manner (right). (B) Some tumor cells are poorly recognized by Vγ9Vδ2 T cells (left). Upon treatment with aminobisphosphonates (n-BP), increased levels of phosphoantigens are produced that induce changes in the membrane mobility of CD277 molecules leading to activation of Vγ9Vδ2 T cells (right). Professional illustration by Alice Y. Chen.
superfamily. By generating a panel of activating or inhibitory anti-CD277 monoclonal antibodies (mAbs), Harly and coworkers uncovered that certain activating anti-CD277 mAbs induced a selective and exclusive activation and proliferation of Vγ9Vδ2 T cells when added to in vitro cultures of peripheral blood lymphocytes, similar to what is seen when phosphoantigens or n-BP are used for in vitro stimulation. On the other hand, they had developed other inhibitory anti-CD277 mAbs that specifically blocked the activation and proliferation of Vγ9Vδ2 T cells in response to phosphoantigens and n-BP. Moreover, the recognition of Vγ9Vδ2-susceptible lymphoma cells was also strongly inhibited by the blocking anti-CD277 mAbs. A series of elegant experiments then addressed the question of how the CD277 molecule contributes to the phosphoantigen-mediated γδ T-cell activation. To this end, they specifically knocked down the expression of different isoforms of the CD277 molecule and found out that the membrane mobility of CD277 was drastically influenced by phosphoantigens through the intracellular part of one particular isoform. Together with additional experiments, the results of this study suggest that activating anti-CD277 mAbs and phosphoantigens modify the CD277 molecule in such a way that the γδ T-cell receptor is triggered on coculture with CD277-expressing cells. It appears that agonistic anti-CD277 mAbs induce changes in the membrane mobility of the CD277 molecules that are somehow sensed by the Vγ9Vδ2 T-cell receptor (see figure panel A). In the case of phosphoantigens generated in tumor cells, particularly after treatment with n-BP, the presented data are in line with the assumption that the intracellularly generated phosphoantigens (ie, IPP) act through the intracytoplasmic domain of one particular isoform.

Together with additional experiments, the results of this study suggest that activating anti-CD277 mAbs and phosphoantigens modify the CD277 molecule in such a way that the γδ T-cell receptor is triggered on coculture with CD277-expressing cells. It appears that agonistic anti-CD277 mAbs induce changes in the membrane mobility of the CD277 molecules that are somehow sensed by the Vγ9Vδ2 T-cell receptor (see figure panel A). In the case of phosphoantigens generated in tumor cells, particularly after treatment with n-BP, the presented data are in line with the assumption that the intracellularly generated phosphoantigens (ie, IPP) act through the intracytoplasmic domain of one particular isoform.

The studies of Harly et al have undeniably discerned a pivotal role of the CD277 molecule for the activation of human Vγ9Vδ2 T cells. This notwithstanding, several issues require further analysis. Most importantly, it is presently unknown how the Vγ9Vδ2 T cells recognize the phosphoantigen (or anti-CD277 mAb)-induced changes of the CD277 surface molecule. The possibility of direct interaction of the Vγ9Vδ2 T-cell receptor with the modified CD277 molecule appears unlikely on the basis of preliminary experiments mentioned in their report. It is also possible that modified CD277 molecule might recruit additional molecules that are then sensed by the Vγ9Vδ2 T-cell receptor. In any case, the key role of CD277 for activation of Vγ9Vδ2 T cells described by Harly et al also provides an explanation as to why murine cells cannot act as presenting cells for phosphoantigen-reactive human Vγ9Vδ2 T cells: there is no CD277 ortholog in rodents.

 Apart from providing novel insights into the mechanisms of phosphoantigen-dependent γδ T-cell activation, this study also opens new translational aspects. Agonistic anti-CD277 mAbs might be useful reagents to boost the antitumor and antilymphoma/leukemia efficacy of human Vγ9Vδ2 T cells. Considering that this population comprises up to 5% of all blood T cells, new strategies targeting the immunotherapeutic potential of Vγ9Vδ2 T cells might thus have major clinical implications.

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LYMPHOID NEOPLASIA

Comment on Perry et al, page 2290

A promising new biologic prognostic model in diffuse large B-cell lymphoma

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In this issue of Blood, Perry et al show that immunohistochemical markers for the cell–of-origin and the microenvironment in diffuse large B-cell lymphoma delineate 2 groups with markedly different survival.1

Diffuse large B-cell lymphoma (DLBCL) is the most common lymphoma in all countries and its frequency varies from 35% to 60% of non-Hodgkin lymphomas. In the World Health Organization classification, DLBCL is clinically, biologically, and pathologically heterogeneous because it includes over 20 entities, subtypes, and variants.2 Nevertheless, the 2 most common (~ 85%) subtypes of DLBCL are: (1) those with a germinal center B-cell (GCB) origin and (2) those with a non-GCB origin, most of which have activated B-cell (ABC) phenotype. The non-GCB subtype has a more aggressive clinical course than the GCB subtype.3,4

For optimal patient management, an accurate diagnosis is mandatory. For the next 5 or more years, there is agreement among senior academic lymphoma pathologists that immunohistochemistry–based markers will continue to be most valuable in making an accurate diagnosis. Immunostaining is widely available to most pathologists in the developed world, as well as in large hospitals in big cities in the developing world.

Several papers published in the past decade on the clinical utility of separating DLBCL of GCB subtype from the non-GCB/ABC subtype have yielded conflicting survival results, especially since the addition of rituximab to
CD277 takes the lead in human $\gamma^\delta$ T-cell activation

Dieter Kabelitz