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Leukocyte Membrane Molecules—An Introduction

1.1 HISTORY

In the late 1970s and early 1980s, as immunologists came to appreciate the power of monoclonal antibodies as reagents, large numbers of new antibodies were made, analyzed, and published. It was difficult to know whether two different antibodies were directed against the same antigen. The antigen that was later named CD9 is a good example. Five antibodies were described independently; they shared some features: precipitation of a protein band of 24–26 kD, reactivity with platelets, and non-T-non-B acute lymphoblastic leukemia. There were some apparently important differences: one antibody was described as reacting with 26% of normal blood lymphocytes, whereas the other four were reported to react with less than 2%; only one of the five was reported to react with monocytes and polymorphs; there were differences in reported reactivity with chronic lymphocytic leukemia. Later analysis, under “Workshop conditions” (multiple laboratories examining coded panels of antibodies), demonstrated that the antibodies all reacted with the same antigen. The differences in the individual descriptions reflected differences in technique, antibody affinity, and interpretation.

A small group of immunologists recognized the problem and devised a solution: multi-laboratory blind analysis and statistical evaluation of the results. This solution led to the First International Workshop on Human Leukocyte Differentiation Antigens (HLDA), organized in Paris by Laurence Boumsell and Alain Bernard in 1984 (Bernard et al., 1984). The purpose of this and subsequent workshops was to standardize reagents and, through the use of standardized reagents, to develop an understanding of the structure and function of leukocyte cell surface molecules and their utility as diagnostic and therapeutic targets.

Important tools in this process were a nomenclature (the CD nomenclature) and
the publication of books (Leukocyte Typing I-VII), which contained the conclusions of the workshops and the data on which these conclusions were based.

1.1.1 The Significance of the CD Nomenclature and HLDA Workshops

The CD nomenclature, supported by the HLDA workshops and the Leukocyte Typing volumes, has become accepted universally. All immunology journals and journals in other fields that deal with these molecules use the CD nomenclature. The U.S. Food and Drug Administration (FDA) has mandated the workshops in the sense that antibodies that seek approval as diagnostic reagents against CD molecules must have been validated through the HLDA workshops. The World Health Organization (WHO) and the International Union of Immunological Societies have mandated the CD nomenclature.

Nevertheless, there have been major changes in the nature and importance of the contribution made by HLDA workshops in the years since the first one was conducted. The main business of the early HLDA workshops was to identify new human leukocyte cell surface molecules, using antibodies that had been made by immunizing mice with whole cells or cell membranes. The major tools were analysis of reactivity of antibodies with a variety of cell types and statistical analysis of the resulting expression data. The statistical procedure used to identify antibodies with a high probability of reacting with the same molecule was cluster analysis, hence, the name “Cluster of Differentiation.” Information of a confirmatory nature was often contributed by protein analysis—Western blotting or immunoprecipitation followed by gel electrophoresis. By the third HLDA workshop, antibodies were also being used to identify antigen expressed in cDNA libraries, allowing the cloning of the cDNA and molecular characterization of the antigen. However, antibody was still the primary tool for antigen discovery and characterization.

As molecular technologies improved, new human proteins were increasingly identified by first cloning the gene, either through sequence homology with a gene of animal origin or by searching for genes with sequence similarity to known molecules. With increasing frequency, the antibody was made after the protein had been identified, expressed, and characterized.

The quality and reliability of good molecular data has changed the methodologic focus of the HLDA workshops. The primary focus of HLDA has moved to the functional molecules (the “antigens”); the antibodies are tools used in their study. In the late 1970s and early 1980s, the question behind many studies was “What does my antibody react with?” Today we can focus on much more fundamental questions about intercellular interactions and cell–molecule interactions. The ligands and receptors involved in these interactions still need to be described, detected, and measured, and monoclonal antibodies are still the most powerful tools for achieving an understanding of these interactions.

1.1.2 Why Does HLDA Focus on Surface Membrane Molecules?

The early studies (1970s–1980s) focused on the cell surface simply because that was the major focus of immunology at the time—immunologists wanted to know how cells interacted with other lymphocytes, with endothelium, and with antigen. Without the tools to identify, visualize in tissue, and isolate individual cell types, many of the questions that have engaged immunologists for the last generation could not even be formulated, let alone addressed. Diagnostic distinctions, for example, between different types of leukemia, seemed at the time to be capable of being addressed with cell surface markers. From the start of the
monoclonal antibody era (and indeed earlier, using polyclonal antisera), therapy using antibodies to knock out cancer cells or immune cells (to prevent organ graft rejection) was a major driver of research, and here the targets were clearly cell surface molecules.

Once we had an extensive catalog of cell surface molecules, and the tools with which to study their function, cell signaling became a new front in the development of understanding of the immune system. The myriad intracellular molecules—syk, zap, cbl, and their ilk—cry out for a nomenclature accessible to scientists from other fields. The HLDA considered on several occasions whether to extend the CD system to cover these molecules and decided on several occasions not to. The reasons included an anxiety that if the CD nomenclature covered these many hundreds of additional molecules it would lose its focus and ability to help scientists classify and remember them, and the concern that many reagents were polyclonal and so of uncertain specificity.

Recognition of the significant capacity of intracellular molecules to serve as markers of differentiation stage [see, for example, Marafioti et al., (2003)] and that the workshops could perform a useful task without necessarily allocating CD names, finally led the HLDA Council to include intracellular markers of differentiation (Zola et al., 2005), in a set of major changes.

1.1.3 Are We Nearly There?

Any parent will recognize this question—asked by children at any stage in a journey, including the very early stages. The child has no concept of the length of the journey, so it’s a reasonable question. If the journey of the HLDA is from the early chaos described earlier in this chapter to the goal of having a complete catalog of the leukocyte cell surface molecules, how far do we have still to travel?

We cannot be sure, but the evidence suggests that we are less than halfway there. Estimates of the numbers of surface molecules expressed by one functional category of leukocyte, the T lymphocytes, arrive at a number around 1000 (Zola and Swart, 2003). The number of T cell markers currently known to be on T cell surfaces is 100–200. The T cells are the best characterized leukocytes, so the journey is unlikely to be more advanced in the other leukocyte families.

The estimate of the total number of molecules is based on counting of proteins or messenger RNA species expressed by cells and multiplying by the estimated proportion of expressed proteins that are membrane proteins. Some known proteins that have not been identified on T cells may nevertheless be expressed on T cells. In arriving at the estimated number of molecules, we have not taken into account post-translational modifications. Clearly, there is room for error in either direction. But the conclusion that we are nowhere nearly “there” receives some support from two other lines of evidence.

First, for the recent Eighth HLDA Workshop, we identified 180 known molecules that could qualify for CD status, and half of them were given CD status at the end of the workshop (Zola et al., 2005). This yield is better than any previous HLDA workshop (Fig. 1.1). If we were nearly at the end of the list, we would expect to be seeing diminishing returns.

Second, several proteomics-based discovery projects have found more new molecules than known ones, (Peirce et al., 2004; Nicholson et al., 2005; Loyet et al., 2005; Watarai et al., 2005). Again, there is no evidence of the diminishing returns we would expect to see if we had a near-complete catalog of leukocyte molecules.

The availability of genomic sequence, coupled with recent developments in proteomics technology, indicates that there is a window of opportunity over the next 5–10
years to complete the discovery process. Based on the number of CD molecules known currently that serve as targets for diagnosis and therapy, we can reasonably expect to find many new diagnostic and therapeutic targets among the molecules to be discovered.

### 1.2 STRUCTURE AND FUNCTION

Most molecules that serve as useful markers of differentiation on leukocyte membranes are glycoproteins. There is enormous variation of structure, but there are also recognizable themes and motifs. Structural features of proteins are summarized in Boxes 1.1 to 1.4. Function depends on structure, so structure can predict function to a significant degree. Determination of the three-dimensional structure of a molecule depends on X-ray diffraction analysis or nuclear magnetic resonance studies, which are complex and require time and resources. Only a few leukocyte membrane molecules have their structure analyzed at this level, but as the number of “solved” structures increases, our ability to predict the tertiary structure of proteins from amino acid sequence improves.

Thus, given an amino acid sequence, usually translated from a DNA sequence, we can predict the structure, and at least some likely functions, for any protein. Structural prediction is most effectively done with Web-based resources, which will be described in Chapter 2. It is important to remember that such structures, and derived functions, are predictions and will almost certainly be wrong in some aspect.

The functional requirements of immune cells are diverse, and the molecules that mediate surface interactions are correspondingly diverse in structure. Nevertheless, several molecular themes recur, and classifying the molecules according to their molecular structure is helpful in understanding both structure and function.

#### 1.2.1 Protein Domains

Protein domains (see Box 1.1) are distinct subunits of proteins that are associated with a particular molecular function, such as protein–protein interactions or kinase activity. One characteristic of domains, which is used in identifying them, is that interactions between residues within a domain are generally stronger than interactions between residues in different domains. Domains usually occur as individ-
Domain: A protein domain is a distinct subunit of a protein. Many proteins are composed of several domains, separated by short or long sequences that may act as “hinge” regions or “spacers.” Domains have characteristic structural features, and about 20 domains have been described (Table 1.1). Domain structure dictates domain function. It is believed that there are domain structures still to be discovered.

Module: The term “module” can be used interchangeably with domain. It tends to be used when describing a domain in a protein that has several different domains—a modular protein.

Motif: Motif is used to describe a shorter sequence than a domain or module, but a sequence associated with a particular structural or functional feature.

Family: Proteins are grouped together in families based on structural similarities. Because the structural features that allow the family relationship to be discerned are largely located in the domains, and many proteins have multiple different domains, a protein may be assigned to several families. The term “family” is applied to the gene as well as the protein.

Superfamily: Superfamilies are broader groupings that include several families that show similarity.

Differences in the intracellular domains can result in proteins with identical or similar extracellular domains having opposite functions. For example, the four TRAIL receptors (CD261, CD262, CD263, and CD264) that are encoded by separate genes all have a single extracellular TNFR domain, but they differ by the presence, absence, or truncation of an intracellular Death domain.

The sequencing of the human genome has lead to the development of programs that can annotate conserved protein domains automatically. These annotations can be used to assign some function to proteins that have not been identified using antibodies. The InterPro database (www.ebi.ac.uk/interpro) (Mulder et al., 2005) provides a single access resource for the major protein domain signature databases.

1.2.2 Protein Families

The classification of proteins into families originally relied on the presence of a shared protein domain and could therefore
<table>
<thead>
<tr>
<th>Domain</th>
<th>Structure</th>
<th>Functions</th>
<th>Example CD Molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABC Transporter</td>
<td>Multiple transmembrane domains coupled to nucleotide binding domain</td>
<td>ATP-dependent transport of substances across membranes</td>
<td>CD243, CD338</td>
</tr>
<tr>
<td>Cadherin</td>
<td>A beta-sandwich formed of seven strands in two sheets. Ca++ ions bind residues from neighboring domains.</td>
<td>Adhesion</td>
<td>CD144, CD324, CD325</td>
</tr>
<tr>
<td>C-type Lectin</td>
<td>Open structure consisting of five beta strands and two alpha helices, with four loops of relatively unstructured sequence.</td>
<td>Calcium-dependent sugar binding</td>
<td>CD62L, CD72, CD141</td>
</tr>
<tr>
<td>CUB</td>
<td>Ig-like beta barrel with four conserved cysteines that probably form two disulphide bridges (C1–C2, C3–C4).</td>
<td>Complement components</td>
<td>CD205, CD303</td>
</tr>
<tr>
<td>DEATH</td>
<td>A homotypic protein interaction module composed of a bundle of six alpha-helices.</td>
<td>Regulation of apoptosis and inflammation through the activation of caspases and NF-kappaB</td>
<td>CD95, CD261, CD271</td>
</tr>
<tr>
<td>EGF</td>
<td>A two-stranded beta-sheet followed by a loop to a C-terminal short two-stranded sheet. The domain includes six cysteine residues that have been shown to be involved in disulphide bonds.</td>
<td>Unknown</td>
<td>CD62L, CD97, CD141, CD339</td>
</tr>
<tr>
<td>Fibronectin 2</td>
<td>The domain contains four conserved cysteines involved in disulphide bonds and is part of the collagen-binding region of fibronectin.</td>
<td>Protein binding</td>
<td>CD205, CD206, CD222</td>
</tr>
<tr>
<td>Fibronectin 3</td>
<td>Seven beta-strands modeled to fold into antiparallel beta-sandwiches with a topology that is similar to immunoglobulin constant domains.</td>
<td>Protein binding</td>
<td>CD122, CD130, CD171</td>
</tr>
<tr>
<td>GPCR</td>
<td>Seven-transmembrane helices, G-protein binding loop.</td>
<td>Receptors</td>
<td>CD97, CD195, CD294</td>
</tr>
<tr>
<td>Ig-like</td>
<td>The fold consists of a beta-sandwich formed of seven strands in two sheets, usually stabilized by intradomain disulfide bonds.</td>
<td>Protein–protein and protein–ligand interactions</td>
<td>CD3, CD19, CD50, CD80, CD171, CD226, CD300a</td>
</tr>
<tr>
<td><strong>LDLR</strong></td>
<td>Complement-like cysteine-rich repeats</td>
<td>Bind multiple ligands</td>
<td>CD91</td>
</tr>
<tr>
<td><strong>Link</strong></td>
<td>Two alpha helices and two antiparallel beta sheets arranged around a large hydrophobic core similar to that of C-type lectin.</td>
<td>Hyaluronan(HA)-binding region</td>
<td>CD44</td>
</tr>
<tr>
<td><strong>LRR</strong></td>
<td>2–45 motifs of 20–30 amino acids in length that generally folds into an arc or horseshoe shape.</td>
<td>Protein–protein interactions</td>
<td>CD180, CD281</td>
</tr>
<tr>
<td><strong>Protein tyrosine kinase</strong></td>
<td>Cytoplasmic protein kinases.</td>
<td>Catalyses the addition of a phosphate group to a tyrosine residue</td>
<td>CD117, CD136, CD167a, CD331</td>
</tr>
<tr>
<td><strong>SCR</strong></td>
<td>Domain contains four repeats of a well-conserved region, which spans 115 amino acids, and contains six conserved cysteines</td>
<td>Protein binding</td>
<td>CD5, CD6, CD163</td>
</tr>
<tr>
<td><strong>Semaphorin</strong></td>
<td>A variation of the beta propeller topology, with seven blades radially arranged around a central axis. Each blade contains a four-stranded (strands A to D) antiparallel beta sheet.</td>
<td>Detect and respond to chemical signals</td>
<td>CD100, CD108, CD136</td>
</tr>
<tr>
<td><strong>Sushi</strong></td>
<td>The structure is based on a beta-sandwich arrangement; one face made up of three beta-strands hydrogen-bonded to form a triple-stranded region at its center and the other face formed from two separate beta-strands.</td>
<td>Protein binding</td>
<td>CD21, CD25, CD35, CD62L</td>
</tr>
<tr>
<td><strong>TNF</strong></td>
<td>Central beta sheet.</td>
<td>Trimeric cytokines</td>
<td>CD153, CD154, CD178</td>
</tr>
<tr>
<td><strong>TNFR</strong></td>
<td>Repeats containing six conserved cysteines, all of which are involved in intrachain disulphide bonds.</td>
<td>Binding of TNF ligand domains</td>
<td>CD30, CD40, CD95</td>
</tr>
<tr>
<td><strong>Phosphotyrosine phosphatase</strong></td>
<td>Cytoplasmic tyrosine-specific protein phosphatases.</td>
<td>Catalyzes the removal of a phosphate group attached to a tyrosine residue</td>
<td>CD45, CD148</td>
</tr>
</tbody>
</table>
**Box 1.2. CARBOHYDRATE STRUCTURES**

Carbohydrates may be attached to protein (glycoprotein) or lipid (glycolipid).

The monosaccharides that occur most often in leukocyte antigens are as follows:

- N-acetyl galactosamine (GalNac)
- Sialic acid (N-acetyl neuraminic acid)
- Glucose (Glc)
- Galactose (Gal)
- Mannose (Man)

Monosaccharides are linked together to form oligosaccharides or polysaccharides.

Each linkage is described as follows: Glc-alpha1-3GalNac means glucose is linked from its number 1 carbon to carbon 3 of N-acetyl galactosamine. The formation of the link changes the #1 carbon of the glucose from a state that allows free rotation to a state in which it is fixed in one of two mirror-image orientations alpha or beta. In the example given, it is the alpha orientation.

Glycosaminoglycans (GAGs) are polysaccharides that are chains of disaccharides or longer oligosaccharides that include an amino sugar. The amino sugar is generally glucosamine or galactosamine, and it is often acetylated or sulfated. The major GAGs found in leukocyte membrane molecules are chondroitin sulfate and heparin or heparan sulfate.
Integral membrane proteins cross the lipid bilayer. In the simplest form, an integral membrane protein consists of an extracellular region, a membrane spanning region, and a cytoplasmic region. The membrane spanning region is generally an alpha helix consisting largely of hydrophobic amino acids, allowing it to be stable in the hydrophobic lipid environment. Some proteins include a charged residue in the membrane-spanning region, which interacts with charged residues on other proteins.

Type I integral membrane proteins have the N-terminus outside the cell and the C terminus in the cytoplasm. A signal sequence is cleaved from the N terminus before export to the cell surface.

Type II integral membrane proteins have their C terminus outside the cell and the N-terminus in the (generally short) cytoplasmic sequence. The membrane-spanning sequence is uncleaved but resembles a signal sequence for protein secretion.

Type III integral membrane proteins pass the membrane several times. Examples are found ranging from two to seven passes through the membrane.

Type IV integral membrane proteins are multipass proteins, like type III proteins. However, in type IV proteins, the proteins form a hydrophilic pore through the membrane.

Type V refers to proteins that do not pass through the membrane but are attached to it by their C-terminus via glycosyl-phosphatidylinositol (a GPI link). In more detail, the C-terminal amino acid is linked to ethanolamine, which is in turn linked through a phosphodiester link to a short string of sugars that link via myoinositol through a phosphodiester to glycerol and thence to the membrane fatty acids.

In addition, proteins may be linked to the membrane through other proteins, via salt bridges, disulphide bonds, or non-covalent interactions.

Proteins that do not pass through the membrane, or have a short cytoplasmic sequence devoid of signal-initiating motifs, may nevertheless transducer signals to cells through interaction with other proteins that do have signaling capacity. Receptors often consist of two or more protein chains, where one binds the ligand and the other transmits the signal.

The assignment to a family is usually based on a single domain that can be associated with a common protein function. There have been recent efforts to formalize the assignment of protein to families, based on aspects of the evolutionary relationship between proteins (Wu et al., 2004).

The members of those families that are more closely related tend to occur in clusters in the genome, such as the KIR family of immunoreceptors, which are located on
<table>
<thead>
<tr>
<th>Family</th>
<th>Domain</th>
<th>Functions</th>
<th>Example CD Molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADAMs</td>
<td>Metallopeptidase</td>
<td>Matrix degradation</td>
<td>CD158a, CD158c</td>
</tr>
<tr>
<td>Cadherins</td>
<td>Cadherin</td>
<td>Adhesion</td>
<td>CD144, CD324, CD325</td>
</tr>
<tr>
<td>IgSF</td>
<td>Ig-like, ICAM</td>
<td>Protein–protein and protein–ligand interactions</td>
<td>CD3, CD19, CD80, CD171, CD226, CD300a, CD50, CD242</td>
</tr>
<tr>
<td>Integrins</td>
<td>Heterodimer of alpha and beta chains</td>
<td>Adhesion</td>
<td>CD18, CD41, CD61, CD49a, CD103</td>
</tr>
<tr>
<td>LILR</td>
<td>Ig-like</td>
<td>Immunoreceptors</td>
<td>CD85-family, CD335</td>
</tr>
<tr>
<td>MS4A</td>
<td>4-transmembrane domain</td>
<td>Various</td>
<td>CD20</td>
</tr>
<tr>
<td>Rhodopsin GPCR</td>
<td>7-transmembrane domain</td>
<td>Receptors</td>
<td>CD191, CD193, CD294</td>
</tr>
<tr>
<td>Secretin GPCR</td>
<td>7-transmembrane domain</td>
<td>Receptors</td>
<td>CD97</td>
</tr>
<tr>
<td>Selectins</td>
<td>Lectin, EGF, sushi</td>
<td>Adhesion</td>
<td>CD62I, CD62E, CD62P</td>
</tr>
<tr>
<td>SEMA</td>
<td>Semaphorin, plexin</td>
<td>Cell migration</td>
<td>CD100, CD108</td>
</tr>
<tr>
<td>SIGLEC5s</td>
<td>Sialic acid-recognizing Ig-superfamily lectins</td>
<td>Adhesion</td>
<td>CD22, CD33, CD170</td>
</tr>
<tr>
<td>Tetraspanins</td>
<td>Four transmembrane domain</td>
<td>Various</td>
<td>CD9, CD37, CD81, CD151</td>
</tr>
<tr>
<td>TNFRSF</td>
<td>TNFR</td>
<td>Binding of TNF ligand domains</td>
<td>CD27, CD30, CD40, CD95, CD271</td>
</tr>
<tr>
<td>TNFSF</td>
<td>TNF</td>
<td>Trimeric cytokines</td>
<td>CD70, CD153, CD154, CD178, CD252</td>
</tr>
</tbody>
</table>

Note: The domains listed are characteristic domains of the families. The functions are indicative of a function for the family.
Chromosome 19 in humans, or the MS4A family of which CD20 is a member, which is located on chromosome 11. Other families can be spread throughout the genome: There are members of the Ig-like superfamily on each chromosome.

### 1.3 Utility in Research, Diagnosis, and Therapy

The availability of the full human genome sequence (Venter et al., 2001; McPhersen et al., 2001) has changed the world irreversibly. As with all significant knowledge, once we have it, we cannot revert to not having it, and if we ignore it, we risk becoming irrelevant. However, the euphoria that accompanied the publication of the genome sequence was quickly followed by a reassessment of what we still need to know, to make use of the genomic information. Even before completion of the sequence, those involved were turning their attention back to function and hence to proteins (Broder and Venter, 2000).
the rapidly developing field known as proteomics.

Knowing the DNA sequence is a starting point. From the genome we need to define the genes—the coding sequences. Every cell has the same genes (ignoring for the moment processes where parts of the genome are spliced out or mutated during the course of differentiation). What characterizes different cells with different functions is the set of genes that is expressed. So the emphasis shifts from gene sequencing to understanding the protein profile of cells and how it relates to function.

The sequence information is being converted rapidly into a catalog of genes, which can be searched for molecules of likely relevance to the immune system in a variety of ways. We can look for homologs to known human immunoreceptors or, more broadly, for members of molecular families that are known to be used by the immune system. From the sequence information, we can construct probes and use Northern blots, in situ hybridization, microarray technology, or polymerase chain reaction (PCR) to determine what is being expressed in cells of the immune system and what is differentially expressed in different cells or states of the immune system.

Antibodies remain uniquely useful tools in the analysis of expression and function of molecules, particularly surface membrane molecules. Although methods based on mRNA detection are improving in quantitative precision and accuracy, there is no direct and universal relationship between the amount of mRNA for a protein in the cell and the amount of protein actually present, and it is the protein that mediates the function. Flow cytometry has sophistication, precision, dynamic range, and ability to analyze on a single-cell basis that will be difficult to improve on, and it works particularly well with antibodies as probes. Immunohistochemical methods, used together with video image analysis, allow studies of tissue sections, which approach flow cytometry in precision and sensitivity, and additionally provide information on spatial relationships. Several immunoassay formats provide sensitive and precise methods of measuring the concentration of molecules in solution, provided specific antibodies are available.

The ability of antibodies to act as agonists or antagonists facilitates the analysis of the functions of molecules, particularly those on the cell surface. Finally, antibodies can make therapeutic reagents, for example, in cancer (Grillo-Lopez et al., 1999) and in situations where we need to modulate immune reactivity (Nashan et al., 1997).

1.3.1 Major Applications of Antibodies

CD markers provide an outstanding example of the power of antibodies in identification, quantitation, and localization of cells in order to analyze function and disease state. (See Table 1.3 for major applications). Flow cytometry using multiple antibodies conjugated to different fluorescent dyes, beads, or nanoparticles allows the determination of cell lineage and sublineage using several different markers in widely available instruments. For example, T cells are identified by the expression of CD3, and the T cells of the “helper” subset (which interact with antigen-presenting cells using MHC Class II to present antigen) are identified by expression of CD4. Within the CD3/CD4 double-positive subpopulation, antigen-experienced (memory) cells are identified by the expression of CD45RO and can be further subdivided into effector memory and central memory cells using one of several markers. This example of multiparameter analysis uses four antibodies and colors, in addition to two physical parame-
ters, to identify lymphocytes if the analysis is done on whole blood (Rivino et al., 2004). The cells of a particular functional subset are not just identified by this procedure, but they are also counted. Furthermore, by performing the separation in a preparative fluorescence-activated cell sorter, the cells can be isolated for further analysis, for example, by extraction of mRNA for analysis of expression of other molecules (Seddiki et al., 2005).

The application of CD markers to cell localization has become routine in diagnostic immunopathology, as illustrated by the classification of lymphomas using the Revised European American Lymphoma (REAL) classification (www.pathnet.medsch.ucla.edu/educ/lecture/pathrev/lymphoma.htm).

Some of the same antibodies can be used therapeutically, generally by removing unwanted cells such as malignant B cells (CD20, Hiddemann et al., 2005)) or T cells mediating organ transplant rejection (CD3, Shapiro et al., 2005).

The therapeutic applications of antibodies against CD molecules started early with the use of OKT3 to reverse rejection, but there was then a long period where additional successes were few. There has been a resurgence of the field recently, with several notable successes in the clinic that have profoundly changed the treatment of some cancers, and incidentally created billion-dollar markets (Weinberg et al., 2005).

### REFERENCES


