Medical Applications of Leukocyte Surface Molecules—the CD molecules

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Leukocytes are the cells of the immune system and are centrally involved in defense against infection, in autoimmune disease, allergy, inflammation, and in organ graft rejection. Lymphomas and leukemias are malignancies of leukocytes, and the immune system is almost certainly involved in most other cancers. Each leukocyte expresses a selection of cell surface glycoproteins and glycolipids which mediate its interaction with antigen, with other components of the immune system, and with other tissues. It is therefore not surprising that the leukocyte surface molecules (CD molecules) have provided targets for diagnosis and therapy. Among the "celebrities" are CD20, a target for lymphoma therapeutic antibodies which earns $2 billion annually (and makes a significant difference to lymphoma patients), and CD4, the molecule used by the human immunodeficiency virus (HIV) as an entry portal into cells of the immune system. This short review provides a background to the CD molecules and antibodies against them, and summarizes research, diagnostic, and therapeutic applications of antibodies against these molecules.
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LEUKOCYTES IN HEALTH AND DISEASE

The immune system evolved (presumably) because it protects complex organisms from being overwhelmed by infection. In mammals, the immune system is complex, multi-layered, and tightly controlled. Immune responses are directed against foreign but not self targets, and are controlled by feedback inhibition so as to minimize damage to tissue. The immune system consists of a network of organs, cells, and soluble mediators. Inevitably, the system can malfunction, leading to disease.

The cells of the immune system are the white blood cells, the leucocytes. These include a number of major distinguishable populations, such as the dendritic cells which first capture antigen, process it to a form that can be recognized by T lymphocytes, and present it to the T cells. Lymphocytes are a morphologically distinct population, but are functionally heterogeneous. Lymphocytes are divided into B cells, which make antibodies, and T cells, which control B cells and many other aspects of the immune response. T cells can be sub-divided into multiple functional subsets which interact with each other and with other components of the immune system. Immunological memory, which allows rapid recovery from a second or subsequent infection with an organism experienced previously, resides in T cells and B cells.

Gross abnormalities of lymphocytes are associated with certain diseases, such as chronic lymphocytic leukemia, which is a malignant proliferation of a single clone of B cells, or HIV infection, which leads to the depletion of the $CD4^+$ "helper T cell" population. More subtle abnormalities of lymphocytes are associated with many other diseases, including the autoimmune and allergic diseases.

There are a number of excellent Immunology texts available for the reader who wants to delve deeper, for example Mak and Saunders (1).

LEUKOCYTE SURFACE MOLECULES—THE CD MOLECULES

The interactions of leucocytes with their universe—other cells, tissue matrix, and antigen—occur through the cell membrane, and specifically through membrane proteins, glycoproteins, and glycolipids. Specialized cell function is reflected in specialized cell surface composition. For example a B lymphocyte binds antigen through membrane immunoglobulin (Ig), which is characteristic of B cells and is absent from other leucocytes. Furthermore, when antigen binds Ig, complex molecular machinery involving several other membrane proteins (including CD79, CD19, CD81, and CD21) comes into play. This complex transduces activation signals to the inside of the cell, and regulates activation. Some of these molecules are also restricted to B cells, while CD81 mediates a similar function in T cells.

The characterization and naming of leukocyte surface molecules has been the responsibility of an organization formerly called Human Leukocyte Differentiation Antigens (HLDA) and more recently re-

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named Human Cell Differentiation Molecules (HCDM). This organization devised the CD nomenclature and publishes periodic reports on human cell surface molecules (2–9). There are currently some 500 characterized leukocyte cell surface molecules, many of them with CD names. It has been estimated that there may be 2,500 leukocyte cell surface molecules in total (10). Although most of these have yet to be characterized and named, the complete set of leukocyte surface molecules will be referred to in this article as CD molecules.

Comprehensive databases of CD molecules include the HCDM web site (www.hcdm.org). The reports of the HLDA Workshops provide detailed information on the molecules as they are characterized, and a comprehensive directory of CD molecules is in press (11). Efforts are under way to identify the "missing" CD molecules by proteomic analysis (12).

ANTIBODIES TO CD MOLECULES

While a number of techniques can be used in the study of CD molecules, antibodies are particularly specific, versatile, and powerful reagents (Table 1). Antibodies can be used analytically to label the molecules and hence cells bearing them, allowing, in turn, measurement of the amount of a CD molecule, the number of cells bearing it, as well as the localization of the molecule and cells bearing it in tissue. Antibodies can be used preparatively to purify (or remove) the molecule from serum or a tissue extract, or to purify (or remove) cells bearing it from cell suspensions.

The analytical applications of antibodies lead to diagnostic assays, while the preparative applications have therapeutic counterparts. Finally, antibody against a CD molecule can be used to probe, simulate, or inhibit the function of the molecule, and this also suggests therapeutic applications.

Lists of antibodies against CD molecules are available from a variety of web sites (including www.hcdm.org) and from suppliers of CD antibodies.

ANALYTICAL AND DIAGNOSTIC APPLICATIONS OF CD ANTIBODIES

Figure 1 shows some analytical data on lymphocytes in a sample of blood. The analysis shows the proportions of T cells, B cells and two major functional subsets of T cells. This is a healthy control sample, but this type of analysis has a number of diagnostic applications which are used routinely in hundreds of pathology laboratories daily. Patients with immune deficiencies may lack one or more lymphocyte types. In acquired immune deficiency due to HIV infection (AIDS), the CD4 cells are attacked, and counts of CD3 or CD4 cells are performed frequently to monitor disease, make treatment decisions, and monitor the effectiveness of therapy. Patients with B cell leukemia will have elevated numbers of B cells and a corresponding fall in the proportion of T cells.

A different example of a diagnostic test based on a CD molecule is the use of CD64. CD64 expression on neutrophils is increased within hours by inflammation or tissue damage. A kit is available from IQ Products (www.iqproducts.nl) which facilitates the analysis of neutrophil CD64 and is marketed for the diagnosis and monitoring of sepsis.

The use of additional CD antibodies allows a more detailed analysis of cells and their probable function; for the most part, these provide information that can help build a picture of disease processes but are, as yet, not well enough established to be accepted as diagnostic tests. For example, there has been a recent surge in interest in cells called regulatory T cells (Tregs), which are thought to be deficient in number or function in autoimmune disease and allergy, and overrepresented or overactive in patients with malignancies that are not being controlled by the immune system. Thus Treg numbers (21) and function (22) have been described as deficient in the autoimmune disease type 1 diabetes. Treg identification is an area of active research (14), and it is likely that we do not yet have the best markers for Tregs.

We can anticipate that many new and more discriminating diagnostic assays will emerge as the full complement of CD molecules is characterized and antibodies are available to numbers of laboratories studying pathophysiology. These will go beyond answering the diagnostic question, "What disease does this patient

Table 1. Clinical applications of monoclonal antibodies

Antibody application	Clinical equivalent	Examples	Reference
Molecular identification and quantitation	Diagnostic pathology	Immunoassay of serum analytes	13
		Flow cytometric enumeration of functional T cell subtypes	14
		Immunohistochemistry	15
Binding and removal of molecules or cells	Antibody therapeutics	Antitoxins	16
		Anti-TNF to dampen inflammation	17
		Anti- CD20 to treat lymphoma	18
		Anti-microbials	19
Agonistic and antagonistic function of antibodies	Antibody therapeutics	Immunostimulatory antibodies in cancer therapy	20

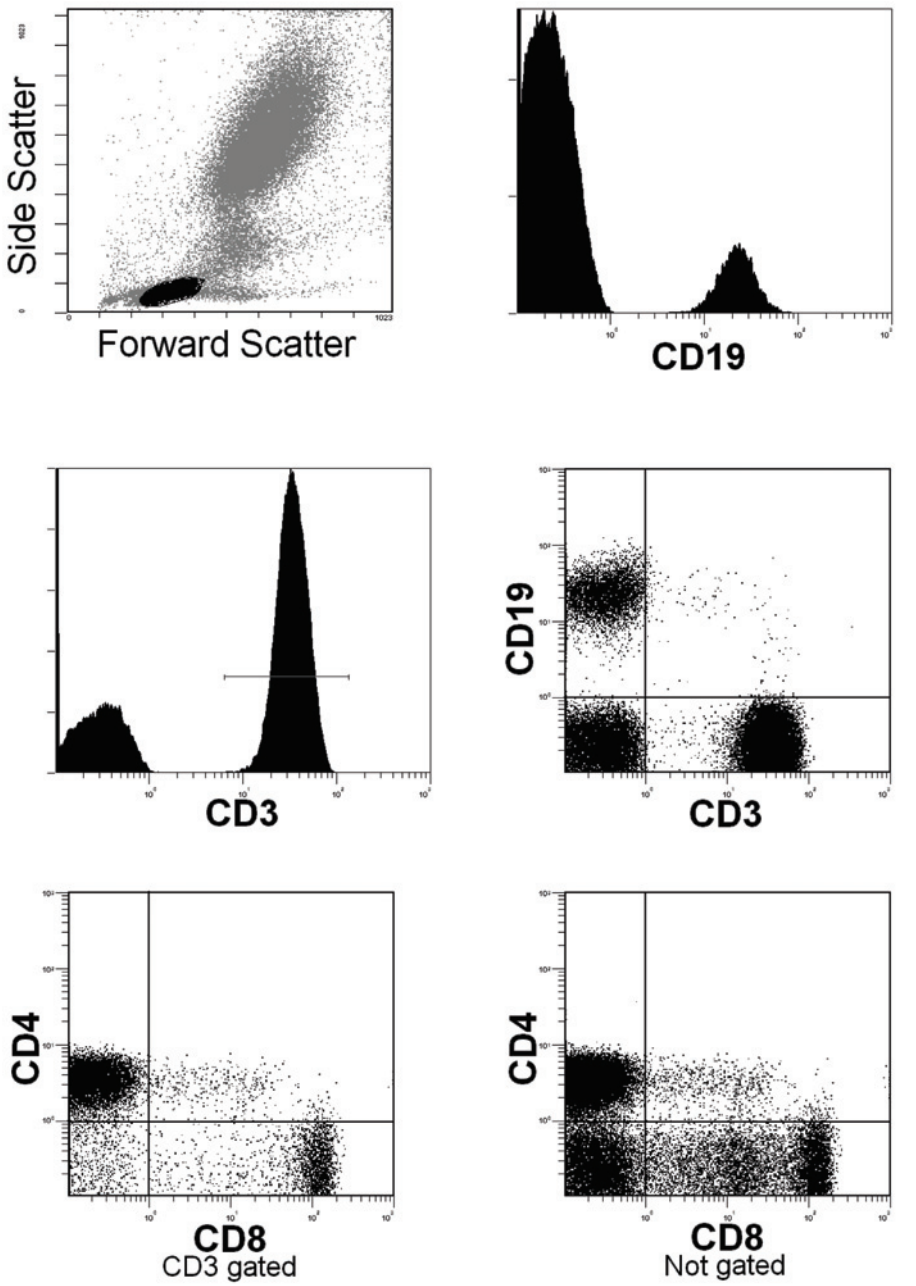

Figure 1. Flow cytometric analysis of blood cells to count B cells, T cells, and the two major T cell sub-sets, CD4 and CD8 T cells. The upper left panel shows a plot of forward (low-angle) light scatter against side (90°) light scatter, proportional to size and internal complexity of cells respectively. This plot allows the selection of the lymphocyte population (black) from the other blood cells (gray). Subsequent panels show fluorescence signals from the lymphocytes only ("gated" on lymphocytes by dual scatter). Top right: CD19, a marker for B cells. Middle left: CD3, a marker for T cells. Middle right: dual color plot of CD3 against CD19, showing T cells, B cells, and cells which are neither T cells nor B cells, with a very small number of cells reacting with both reagents. The bottom panels show plots of CD 8a against CD8, looking at only the T cells ("gated" on CD3–positive cells, left) or all lymphocytes (gated only by dual scatter, right). CD4 and CD8 expression are seen to be largely mutually exclusive. The flow cytometer provides precise counts of the relative numbers of cells in each population.

have?" to the more useful question, "What is the best treatment for this patient at this time?"

THERAPEUTIC APPLICATIONS OF CD ANTIBODIES

Antibodies have been used therapeutically for many years, starting (as far as we know) with the use of horse antisera against bacterial toxins by Emil von Behring and Shibasaburo Kitasato. In the field of CD molecules, an early success was the use of OKT3, a CD3 antibody, to reverse organ graft rejection. With this exception, CD antibodies failed for many years to live up to expectations. The reasons are interesting, but the turn around is much more interesting. Monoclonal antibodies, particularly against CD molecules and related components of the immune system, are currently having a major impact on a number of diseases and perhaps a bigger impact on the biotechnology industry. The number of antibodies undergoing clinical trial and late stage preclinical evaluation is even more impressive. A recent highly-publicized adverse event (23) reminds us of the dangers, and there have been other unsuccessful trials, but the successes are impressive.

Tables 2 and 3 list some of the CD antibodies in current clinical use. About 200 antibodies are undergoing clinical evaluation (46), while an industry web site provides a list of many antibodies undergoing pre-clinical testing (PharmaProjects Database PJB Publications, available at http://www.pjbpubs.com/pharmaprojects/index.htm).

CURRENT LIMITATIONS AND WHERE THE FIELD MIGHT GO

After a long period of very slow progress, the therapeutic applications of monoclonal antibodies are expanding at an explosive pace. What are the limits? First, expansion is limited by the pool of available diseases and patients in economies that can afford such relatively expensive therapeutics. We may anticipate that advances will come in the form of, for example, a better CD20 (47), which will be good for patients but will

Table 2. Monoclonal antibodies against CD molecules in clinical use for therapy of cancer

Specificity	Target disease[a]	Antibody	Antibody type	Reference
CD20	Lymphoma[b]	Rituximab	Humanized	24
		Zevalin	Radioconjugate	25
		Bexxar	Radioconjugate	26
CD19	Lymphoma	B4	Ricin immunotoxin	27
CD22	Lymphoma	LymphoCide	Radioconjugate	28
CD52	Lymphoma, leukemia	Campath 1H	Humanized	29
CD25	Human T-lymphotropic virus type I (HTLV-I)-induced malignancy	Zenepax	Humanized	30
		Simulect	Chimeric	31
CD33	Acute myeloid leukemia	Mylotarg	Calicheamicin conjugate	32
CD15	Acute myeloid leukemia	PM-81	Murine IgM	33
HER2 (CD340)	Breast cancer	Herceptin	Humanized	34
		Pertuzumab	Humanized	35
MUC-1 (CD227)	Ductal breast tumors	BrE-3	Humanized	36
Transferrin receptor (CD71)	Various malignancies	42/6	Murine IgA	37
Carcinoembryonic antigen (CD66e)	Colorectal cancer, other epithelial tumors including lung, breast	Anti-CEA	Chimeric	38

compete with the existing CD20 therapeutics, thus slowing the rate of growth. Cost is a limitation that will always be with us. Unlike small-molecule chemical "drugs," antibodies will always be expensive to make, and cut-price "generics" will still be expensive. It is not yet clear whether fully-human antibodies will be limited by anti-idiotypic responses—these will undoubtedly occur, but may not be limiting (48).

Nevertheless, there are still many opportunities in cancer, transplantation, inflammation and autoimmune diseases, and the infectious diseases. Whereas antibody-mediated therapies once were seen as having a limited application, of interest only until a relatively cheap chemical drug was available, antibodies are now seen as having significant advantages over chemical drugs, because of their specificity. This does not mean an absence of side effects (23), but it should be possible to predict therapeutic and undesirable effects from knowledge of the biological mechanisms addressed by the antibodies.

Successful as therapeutic antibodies have been in recent years, we are still working with a small number of target molecules, and a limited set of effector mechanisms. Therapeutic effects are impressive, but far from complete. The CD20 antibody in current clinical use is effective for only a proportion of CD20-positive lymphomas (47), and the several anti-inflammatory treatments based on antibodies also address only a proportion of patients successfully. The use of immunotoxins (49) may be part of the answer, while understanding and utilizing the full range of immunological mechanisms available to an antibody (50,51) will also help. If our estimate that there are many more CD molecules to be discovered (10) is correct, we will have more targets from which to chose. When we have a better understanding of the mechanisms involved when antibodies interact with cells, we may be able to control tumors and adverse immune reactions more effectively than we can now.

Table 3. Monoclonal antibodies against CD molecules in clinical use or undergoing trial for therapy of immune system disorders

Specificity	Target disease	Antibody	Antibody type	Reference
CD3	Transplant rejection	Muromonab	Murine antibody	39
CD4	Psoriasis	Imuclone	Humanized	40
CD11a	Psoriasis	Efalizumab	Humanized	41
CD20	Rheumatoid arthritis	Rituximab	Humanized	42
CD25	Transplant rejection	Basiliximab, Daclizumab	Chimeric Humanized	43
CD52	Inflammation	Campath-1	Humanized	44
CD154	Autoimmune disease	IDEC-131	Humanized	45

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