The next cluster of differentiation (CD) workshop

Timothy A. Springer

One may participate in the next workshop by submitting monoclonal antibodies (mAbs) that react with molecules on the surface of human leukocytes, platelets or endothelial cells, by carrying out investigations on the mAb panels sent out by the workshop, or by attending the concluding meeting.

THE Workshop on Human Leukocyte Differentiation Antigens organizes international exchanges of mAbs, cooperative research on cell-surface molecules, and the CD nomenclature. Plans have just been laid for the fifth workshop, and I have been asked by the organizing committee to summarize these for members of the scientific community so they may understand how this important scientific undertaking works, how to participate, and how new features such as sections on adhesion structures, cytokine receptors, and endothelial cells broaden the scope of the workshop. This broadening of scope reflects an increasing emphasis in the workshops on the function of cell-surface molecules. There is no doubt that these workshops have substantially contributed to the extremely rapid advances that mAb technology has enabled not only on the serology but also on the molecular and cell biology of leukocyte cell-surface molecules. Extra dimensions about these molecules are revealed because scientists come together that are studying the same molecule under different guises — under different names, often on different cell types, and commonly from different points of view — as a surface antigen, a leukocyte subpopulation or malignant cell marker, a protein structure, a cDNA sequence, a receptor, an adhesion molecule, an enzyme, or a functional entity important in antigen-specific responses, host defence or homeostasis. The exchange of reagents — in the last workshop over 1,100 mAbs were analysed and over 500 laboratories participated — provides rich cross-fertilization for the field and encourages cooperation that extends beyond the workshop.

CD nomenclature

The CD nomenclature has been widely adopted, relieving the previous babel, yet few researchers know that CD stands for "cluster of differentiation", let alone the reference system and procedures that lie behind the nomenclature. Since the inception of the workshops, the primary criterion for grouping together mAbs that react with the same cell-surface structure has been reactivity with a panel of cell types. The percentage of cells positive for each antibody by indirect fluorescent staining and flow cytometry is determined for purified leukocyte subpopulations and cell lines. A matrix of distances between antibodies, that is, the absolute difference in percentage reactivity for each pair of antibodies averaged over all cell targets, is used to construct a dendrogram revealing which antibodies have the most similar patterns of reactivity, or in other words form "clusters of differentiation". Reference mAbs are thus chosen that define each CD. Molecular characterization of the target antigen has revealed excellent agreement with this clustering method, and is now required for a final CD assignment. The CD designation originally referred to mAbs. This was considered an operational expedient, because in some cases CDs were assigned before molecular structures were known, and some structures are complex, involving carbohydrate determinants or alternative mRNA splicing. However, the CD designation has now become widely used to refer to the structures that are defined by CD mAbs.

How the workshop works

The workshop is divided into sections*, to increase efficiency and spread out the burden among the organizers of handling and distributing the large number of mAbs that are studied. The sections will be forums where antibodies will be gathered that are of interest to people working on a specific cell type or specific class of surface molecules. The antibodies do not have to be specific for a given cell lineage, but must react with a given cell lineage in the case of sections that focus on a specific cell type. Each section will sponsor a main panel containing unclustered mAbs and a few clustered reference mAbs. Where interest warrants, additional "CD panels" with collections of mAbs putatively reacting with the same, previously clustered CD molecule will be prepared. Some of the CD panels will be organized as "satellite workshops" by outside volunteers; those interested in a specific CD should apply to the workshop organizing committee and obtain its endorsement.

The first step in the workshop (January 1992) will be a call for antibodies. Researchers are invited to submit information on antibodies they think would be interesting to study, particularly those that recognize previously unclustered antigens. Any antibody that reacts with leukocytes, platelets or endothelial cells, or activated or tumour cells of these lineages, is eligible. Careful selection of submitted antibodies is important to maximize the efficiency of the workshop, that is, the ratio of new CD assignments to antibodies analysed. This means that only well characterized mAbs can be accepted. Characterization should be both as to cell distribution and the structure or function of the target antigen; if only cell distribution is known, it should be carefully compared to that found with previously clustered mAbs using the Leukocyte Typing Database (see below). The organizers will also solicit submissions of specific mAbs; a preliminary tally identified over 50 cell-surface structures that have been cloned or identified with mAbs that as yet do not have CD assignments. At least two mAbs, from different laboratories, are required for a CD assignment, so information on related mAbs is encouraged when possible.

The next step in the workshop will be distribution of the mAb panels. Each section will aliquot the submitted mAb into 100–200 replicates of each panel, each containing roughly 100–200 mAbs. Panels will be sent to laboratories that volunteer to help with clustering, or carry out other types of scientific or clinical investigations. Those wishing to receive panels must submit a protocol describing the planned experiments. Priority will be assigned based on the contribution to the clustering and scientific goals of the workshop. Clustering studies may utilize (1) the traditional technique of immunofluorescent flow cytometry (2) immunostaining of tissue sections, a powerful method provided that standardized methods for reporting the staining can be incorporated for cross comparison between laboratories (3) immunoprecipitation (4) reactivity with products of transfected genes and (5) novel methods to be proposed. Basic science studies and clinical investigations using the antibodies can take many forms that are limited only by the imagination. To ensure that both submitters and testers of mAbs benefit in this collegial workshop

enterprise, there is an obligation to report results at the meeting that concludes the workshop, before they are communicated elsewhere. Selected contributions will be chosen for chapters in the workshop book.

Data that are gathered by researchers will be communicated back to the sections distributing mAbs and to a special section for data analysis. This will be analysed so that the new cluster assignments can be presented at the final meeting. This meeting (November 1993) will include presentations of studies carried out in each workshop section and a plenary scientific symposium.

Each workshop is summarized in a book, and for the third and fourth workshops, a computer database and program. The books contain a wealth of information on surface molecules and the mAbs to them[1-4]. All the antibodies studied are listed and indexed, along with their contributors and addresses. This greatly facilitates obtaining mAbs from one's colleagues. The databases contain reactivities of each mAb with each cell type tested, valuable information not found anywhere else[5,6]. The companion programs have many uses. For example, one may find strongly positive cell lines that would be well suited for purification of a particular antigen. Or, the cellular reactivities for one's own newly generated mAb may be added to the database, and the closest matches found with mAbs in the database. A database listing all CD molecules and their defining characteristics could be maintained for access through computer networks, much as is done for nucleic acid and protein sequences.

For further information on how to participate in the workshop, please write to Stuart F. Schlossman, Dana-Farber Cancer Institute, 44 Binney Street, Boston, Massachusetts 02115, USA. □

*Chairman, Stuart F. Schlossman; activation antigens, Roland Schwarting; adhesion structures, Timothy A. Springer; B cells, Thomas F. Tedder; cytokine receptors, Tadamitsu Kishimoto; data management, Stephen Shaw and Wally Gilks; endothelial cells, Michael A. Gimbrone; myeloid cells, Robert F. Todd; NK antigens, Jerome Ritz; platelets, Roy Silverstein; T cells, Laurence Boumsell.

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1. Bernard, A., Boumsell, L., Dausett, J., Milstein, C. & Schlossman, S. F. *Leucocyte Typing: Human Leucocyte Differentiation Antigens Detected by Monoclonal Antibodies* (Springer, Berlin, 1984).
2. Reinherz, E. L., Haynes, B. F., Nadler, L. M. & Bernstein, I. D. *Leukocyte Typing II* (Springer, New York, 1986).
3. McMichael, A. J. *Leukocyte Typing III: White Cell Differentiation Antigens* (Oxford University Press, Oxford, 1987).
4. Knapp, W. *et al. Leukocyte Typing IV: White Cell Differentiation Antigens* (Oxford University Press, Oxford, 1989).
5. Gilks, W. R., Spiegelhalter, D. J. & Cobbold, S. P. *Leukocyte Typing Database III* (Oxford University Press, Oxford, 1988).
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Cellular research briefs

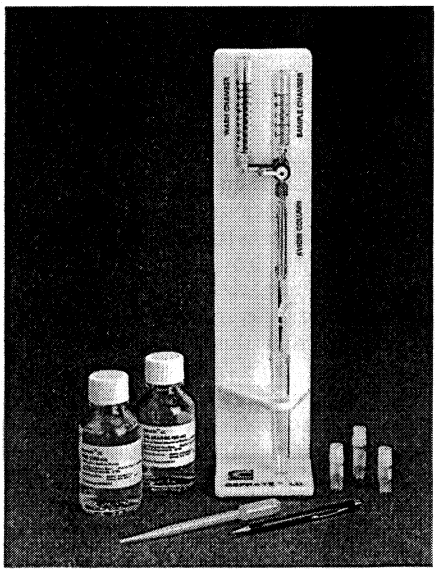
Over 350 companies will be showing off their wares at next week's 31st Annual Meeting of the American Society for Cell Biology to be held in Boston, Massachusetts, USA. Featured exhibits will include a new closed-loop mass cell culture device and an ultrasonic cell disrupter.

USING an indirect CO_2 conductance method, the specially adapted cell in the Malthus 2000 analyser can **detect and enumerate yeasts and moulds,** says Malthus Instruments *(Reader Service No. 101)*. The cell measures conductance changes in a base solution brought about by absorption of CO_2 evolved by the growth of yeasts and moulds in the sample under test. Testing times can be cut down from seven days by traditional testing techniques to less than 24 hours when using the Malthus method, says the company.

Cell separators

CellMicroSieves from BioDesign are a **filter matrix of woven nylon filaments** specially formulated to be inert for biological research *(Reader Service No. 102)*. Available in 11 different pore sizes ranging from 5 μm to 200 μm, CellMicroSieves are designed to provide a useful laboratory tool for the rapid, yet gentle, isolation of cells, organelles, or cellular debris. These specific-sized porous screens can be used to separate cells based on size, collect either cells or media without centrifugation, isolate single cells from dissociated tissue, or rapidly pass media in a bioreactor. As CellMicroSieves can be steam sterilized, they are reusable. Sheet, roll or filter-disk forms of CellMicroSieves are available.

To meet the increasing research interest in haematopoiesis and stem cells, and to facilitate the rapid selection of these rare cells from heterogeneous cell populations such as bone marrow, CellPro has developed the Ceprate LC (CD34) **laboratory cell separation system** *(Reader Service No. 103)*. The Ceprate LC kit is a disposable cell separation system that utilizes avidin–biotin immunoaffinity to separate and enrich progenitor cells ($CD34^+$) more quickly and easily than other currently available techniques, CellPro says. The system contains monoclonal antibodies, solutions, an avidin-gel column and other materials needed to purify those stem cells that are designated $CD34^+$. Each kit can process up to 500 million cells in less than 3 hours, providing high purities and yields of $CD34^+$ cells, CellPro says. The method is designed to provide a greater than 50-fold enrichment of colony forming cells that are able to differentiate into unipotent and multipotent cells (BFU-E, CFU-GM and CFU-MIX). The Ceprate LC system has also been used to select lymphoid subsets and appears to have broad applicability for selecting other cell populations. CellPro is using similar technology to develop a a fetal cell concentrator. As part of a special introductory offer lasting until 31 December, CellPro is offering three Ceprate LC kits for $499.50 (US).



$CD34^+$ cell selection system.

Growth areas

With its space-saving design, the CellCube System developed by Costar represents a new concept in the **mass culturing of anchorage-dependent cells** *(Reader Service No. 104)*. Each CellCube is an integral, self-contained culture module that is comprised of parallel tissue culture-treated polystyrene plates interspersed with media flow channels. Three sizes are available with growth areas of 21,250 cm^2, 42,500 cm^2 and 85,000 cm^2. To illustrate the compact nature of the system, a CellCube module equivalent to