CD molecules 2005: human cell differentiation molecules

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The immune system works through leukocytes interacting with each other, with other cells, with tissue matrices, with infectious agents, and with other antigens. These interactions are mediated by cell-surface glycoproteins and glycolipids. Antibodies against these leukocyte molecules have provided powerful tools for analysis of their structure, function, and distribution. Antibodies have been used widely in hematology, immunology, and pathology, and in research, diagnosis, and therapy. The associated CD nomenclature is commonly used when referring to leukocyte surface molecules and antibodies against them. It provides an essential classification for diagnostic and therapeutic purposes. The most recent (8th) Workshop and Conference on Human Leukocyte Differentiation Antigens (HLDA), held in Adelaide, Australia, in December 2004, allocated 95 new CD designations and made radical changes to its aims and future operational strategy in order to maintain its relevance to modern human biology and clinical practice. (Blood. 2005;106:3123-3126)

Introduction

The Human Leukocyte Differentiation Antigens (HLDA) Workshops were initiated[1] to bring order to the chaos that existed in the early 1980s, as immunologists generated large numbers of monoclonal antibodies reactive with leukocyte cell-surface molecules, each with different associated nomenclatures. In the absence of comparative studies it was often impossible to tell if the same molecule was recognized by more than one antibody. The approach of the workshops was to code antibodies and then send them to multiple participating laboratories for blind analysis against multiple cell types. The data were collated and analyzed by the statistical procedure of "cluster analysis." This analytical method identified clusters of antibodies with very similar patterns of binding to leukocytes at various stages of differentiation: hence the "cluster of differentiation" (CD) nomenclature. This provided a common nomenclature that allowed the scientific community to communicate results in a universal language. The full list of CD molecules may be accessed through the 8th HLDA Workshop (HLDA8) website (www.hlda8.org), which will be replaced by a comprehensive web-based database providing links to protein and gene web-based databases (I.N. and V.H., in preparation).

In the early workshops, the raw material comprised the large numbers of submitted antibodies against unknown molecules. Statistical cluster analysis, together with protein biochemical techniques such as immunoprecipitation, then provided the major tools for identifying the new molecules defined by workshop antibodies. Many of the molecules used every day by immunologists, hematologists, and pathologists were discovered this way, including CD3, CD4, CD8, and CD20. In addition, the workshops provided a forum for the study of function and distribution of molecules and epitopes, and catalyzed the development of an industry providing reagents for research, diagnosis, and now therapy.[2]

However, as molecular biologic techniques grew in power, new molecules present on leukocytes were increasingly identified via gene cloning. Antibodies began to be made after the

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molecule had been cloned, rather than before. Monoclonal antibodies remain useful for research and diagnosis—and are of increasing importance for therapy—but they no longer represent the primary tool for discovery of new proteins (in contrast to glycolipid and carbohydrate epitopes, where antibodies remain the major tool for identifying new molecules).

While these changes progressed, HLDA workshops have continued to be held in a 4-year cycle, with the major aim of allocating CD numbers to well-characterized molecules against which good-quality antibodies are available. However, there has been increasing debate, within as well as outside the HLDA organization, on how appropriate this role may be in the era of molecular proteomics. These discussions culminated in agreement at the meeting of the HLDA Council in December 2004 to make radical changes, including a change in name as well as aims and methods. These are summarized at the end of this article.

Materials and methods

HLDA8 adopted the aims and methodology established at previous workshops (since the changes detailed at the end of this article were not agreed on until after the meeting in December 2004). The major aims were (1) to assign CD names to molecules for which good-quality antibodies (at least one) and good molecular data are available (generally meaning that the molecule has been cloned); (2) to validate new antibodies against existing CD molecules; (3) to evaluate a "blind panel" of new monoclonal antibodies with a view to identifying new molecules by cluster analysis, following the traditional approach described above; and (4) to provide a forum for discussing and developing scientific understanding and practical applications of leukocyte differentiation molecules, including their ligands.

Table 1 lists the 95 molecules that were assigned CD numbers as a consequence of the HLDA8 meeting. The antibodies validated against these "new" CD molecules, as well as newly validated antibodies against preexisting CD molecules, are listed on the HLDA8 website (www.hlda8.org).

A blind antibody panel was evaluated along the lines of previous HLDA blind panels.[3-6] Although a number of pairs and groups of antibodies were found to have very similar reactivity with the panel of cells, we were not able to assign any new CD molecules on the basis of these studies. This was due in part to incomplete biochemical studies, and these are continuing after the completion of the HLDA8 meeting. However, the experience of the nonlineage and adhesion/stromal cell sections, in which a number of antibodies against apparently new molecules turned out to be against existing CD markers, served to emphasise the ever-increasing risk of "rediscovering" known molecules. As a consequence, the HLDA Council decided that, in the future, new CDs deriving from blind-panel analysis would require definitive molecular identification of the target antigen. This may be performed by using the antibody to "expression clone" the corresponding gene or to immunoprecipitate the antigen for mass spectrometric analysis.

The final aim, to provide a forum for discussion and development of the field, was accomplished in part by the 8th HLDA Conference that accompanied the workshop and was held jointly with the Australasian Society for Immunology. This aim will also be achieved by this report and by a number of publications that will follow the workshop. In keeping with current practice, much of the information of archival value (in particular validated antibodies) will be available through the web (www.hlda8.org).

Results

The newly assigned CD numbers are shown in Table 1.

Discussion

The question of whether HLDA has reached the point of diminishing returns is raised after every workshop. However, there is good reason to believe we have so far accounted for just a fraction of the molecules expressed on leukocyte surfaces,[7] and the 95 new designations of HLDA8 support that view. Although there is plenty still to do, are we still going about it in the right way? In recognition of changes in biology generally and in the field served by HLDA, the HLDA Council reached several decisions.

Name change

The acronym "HLDA" will be succeeded by "HCDM" (for "human cell differentiation molecules"). The reasoning behind this name change is as follows. (1) To indicate a break with tradition, while retaining the letters "CD." (2) To maintain the emphasis on molecules of human origin (although the HCDM project will continue to provide leadership and a forum for collaborative studies in other species). (3) To extend the focus from leukocytes to other cell types, recognizing that leukocytes do not act alone. The HLDA project has fostered studies on endothelial molecules for some years, since they interact with cells of the immune system. Furthermore, the HLDA8 meeting included an active section devoted to studies of stromal cell molecules. We therefore believe that the multidisciplinary approach pioneered by previous HLDA workshops can be applied successfully to many other nonhematopoietic human cell types. (4) To broaden the scope from cell-surface molecules to any molecule whose expression reflects differentiation. This would recognize the growing value of intracellular molecules, particularly in immunohistologic studies of tissue sections, as markers of differentiation.

Nomenclature role

The HLDA Council is a subcommittee of the International Union of Immunological Societies (IUIS) Nomenclature Committee, charged with responsibility for generating a consensus nomenclature for leukocyte cell-surface molecules. The HCDM Council will retain this role (subject to the wishes of IUIS) and HCDM will give appropriate consideration to gene nomenclature assigned by the Human Genome Organization (HUGO) Gene Nomenclature Committee. CD numbers will not be allocated as routine practice to all leukocyte surface molecules recognized by newly available antibodies. CD numbers will continue to be allocated to molecules where this appears likely to facilitate communication.

Antibody validation role

Validation of antibodies against known molecules has always been a central aspect of the HLDA organization. This remains an important function: users of commercial antibodies would like the reagents they purchase to be validated by an independent laboratory, since, in spite of technical advances, antibodies are still frequently misassigned by their creators. The HCDM project will therefore undertake this validation role, and do so on a systematic basis. Furthermore, it will not restrict itself to molecules that subsequently receive a CD designation; that is, the nexus between antibody validation and nomenclature will be broken. The HCDM office will maintain a database of all validated hybridoma clones, and will also seek to ensure that validated antibodies are available

Table 1. List of new CD assignments

New CD	Molecule	Gene ID*	Gene symbol
CDw113	PVRL3, Nectin3	25945	*PVRL3*
CD118	LIFR	3977	*LIFR*
CDw156C	ADAM10	102	*ADAM10*
CD159c	NKG2C	3822	*KLRC2*
CD172b	SIRPbeta	10326	*SIRPB1*
CD172g	SIRPgamma	55423	*SIRPB2*
CD181 (was CDw128A)	CXCR1	3577	*IL8RA*
CD182 (was CDw128B)	CXCR2	3579	*IL8RB*
CD185	CXCR5	643	*BLR1*
CDw186	CXCR6	10663	*CXCR6*
CD191	CCR1	1230	*CCR1*
CD192	CCR2	1231	*CCR2*
CD193	CCR3	1232	*CCR3*
CD196	CCR6	1235	*CCR6*
CD197	CCR7	1236	*CCR7*
CDw198	CCR8	1237	*CCR8*
CDw199	CCR9	10803	*CCR9*
CDw218a	IL18Ralpha	8809	*IL18R1*
CDw218b	IL18Rbeta	8807	*IL18RAP*
CD248	TEM1, Endosialin	57124	*CD164L1*
CD249	Aminopeptidase A	2028	*ENPEP*
CD252	OX40L	7292	*TNFSF4*
CD253	TRAIL	8743	*TNFSF10*
CD254	TRANCE	8600	*TNFSF11*
CD256	APRIL	8741	*TNFSF13*
CD257	BLYS	10673	*TNFSF13B*
CD258	LIGHT	8740	*TNFSF14*
CD261	TRAIL-R1	8797	*TNFRSF10A*
CD262	TRAIL-R2	8795	*TNFRSF10B*
CD263	TRAIL-R3	8794	*TNFRSF10C*
CD264	TRAIL-R4	8793	*TNFRSF10D*
CD265	TRANCE-R	8792	*TNFRSF11A*
CD266	TWEAK-R	51330	*TNFRSF12A*
CD267	TACI	23495	*TNFRSF13B*
CD268	BAFFR	115650	*TNFRSF13C*
CD269	BCMA	608	*TNFRSF17*
CD271	NGFR (p75)	4804	*NGFR*
CD272	BTLA	151888	*BTLA*
CD273	B7DC, PDL2	80380	*PDCD1LG2*
CD274	B7H1, PDL1	29126	*PDCD1LG1*
CD275	B7H2, ICOSL	23308	*ICOSL*
CD276	B7H3	80381	NA
CD277	BT3.1	11119	*BTN3A1*
CD278	ICOS	29851	*ICOS*
CD279	PD1	5133	*PDCD1*
CD280	ENDO180	9902	*MRC2*
CD281	TLR1	7096	*TLR1*
CD282	TLR2	7097	*TLR2*
CD283	TLR3	7098	*TLR3*
CD284	TLR4	7099	*TLR4*
CD289	TLR9	54106	*TLR9*
CD292	BMPR1A	657	*BMPR1A*
CDw293	BMPR1B	658	*BMPR1B*
CD294	CRTH2	11251	*GPR44*
CD295	LeptinR	3953	*LEPR*
CD296	ART1	417	*ART1*
CD297	ART4	420	*DO*
CD298	Na^+/K^+-ATPase β3 subunit	483	*ATP1B3*
CD299	DCSIGN-related	10332	*CD209L*
CD300a	CMRF35H	11314	NA
CD300c	CMRF35A	10871	NA
CD300e	CMRF35L1	N/A	NA
CD301	MGL, CLECSF14	10462	*CLECSF14*
CD302	DCL1	9936	NA
CD303	BDCA2	170482	*CLECSF7*
CD304	BDCA4, Neuropilin1	8829	*NRP1*
CD305	LAIR1	3903	*LAIR1*
CD306	LAIR2	3904	*LAIR2*
CD307	IRTA2	83416	NA
CD309	VEGFR2, KDR	3791	*KDR*
CD312	EMR2	30817	*EMR2*
CD314	NKG2D	22914	*KLRK1*
CD315	CD9P1	5738	*PTGFRN*
CD316	EWI2	93185	*IGSF8*
CD317	BST2	684	*BST2*
CD318	CDCP1	64866	NA
CD319	CRACC	57823	*SLAMF7*
CD320	8D6A	51293	NA
CD321	JAM1	50848	*F11R*
CD322	JAM2	58494	*JAM2*
CD324	E-Cadherin	999	*CDH1*
CDw325	N-Cadherin	1000	*CDH2*
CD326	Ep-CAM	4072	*TACSTD1*
CDw327	siglec6	946	*SIGLEC6*
CDw328	siglec7	27036	*SIGLEC7*
CDw329	siglec9	27180	*SIGLEC9*
CD331	FGFR1	2260	*FGFR1*
CD332	FGFR2	2263	*FGFR2*
CD333	FGFR3	2261	*FGFR3*
CD334	FGFR4	2264	*FGFR4*
CD335	NKp46	9437	*NCR1*
CD336	NKp44	9436	*NCR2*
CD337	NKp30	259197	*NCR3*
CDw338	ABCG2, BCRP	9429	*ABCG2*
CD339	Jagged-1	182	*JAG1*

NA indicates not available.

*Gene ID refers to the Entrez gene accession number.[8]

in practice to the research community, whether commercially or otherwise. However, there is a practical limit to the role that HCDM can play in antibody validation. Antibodies can be checked for specificity, and only antibodies that give a clear-cut positive result will be recognized as workshop-validated. However, fitness for particular uses and the provision of antibody at suitable concentrations and in suitable forms (including, for example, conjugates) remains a matter for the companies marketing antibodies.

Dissemination of information

The HCDM laboratories will validate antibodies in a yearly cycle, and the validation of new antibodies and any CD designations accepted by the Council will be posted on the HCDM website at the end of each year. A web-based set of databases will be maintained, including a listing of all antibodies that have been validated through the workshop process, and a database providing basic information for each molecule, together with links to other web-based information resources, allowing quick access to molecular and distribution data.

Conclusion

The HLDA initiative has served immunology, hematology, and pathology well over the 22 years that have elapsed since the first workshop, and it provided much-needed clarity in the chaotic early days of monoclonal antibodies. The order wrought by the pioneers of HLDA studies has supported innumerable research, diagnosis, and therapy initiatives by providing independent confirmation of

antibody specificity, as well as a universal nomenclature scheme. However, advances in technology and knowledge have meant that the HLDA initiative needs to change to maintain its relevance. The recent HLDA8 meeting created 95 new CD assignments, but it also defined radical changes in the aims and methods of the organization. This has positioned the new HCDM organization to play a vital role in future exciting antibody-based studies that will discover and exploit many new functional human proteins.

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