

CD Molecules 2005: Human Cell Differentiation Molecules

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Abstract

The immune system works through leucocytes 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 leucocyte molecules have provided powerful tools for analysis of their structure, function and distribution. Antibodies have been used widely in haematology, immunology and pathology, and in research, diagnosis and therapy. The associated CD nomenclature is commonly used when referring to leucocyte 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 Leucocyte Differentiation Antigens (HLDA), held in Adelaide 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.

Introduction: The HLDA Workshops

The HLDA Workshops were initiated (Bernard et al., 1984¹) to bring order to the chaos that existed in the early 80s, as immunologists generated large numbers of monoclonal antibodies reactive with leucocyte 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 recognised 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 analysed by the statistical procedure of “cluster analysis”. This analytical method identified *clusters* of antibodies with very similar patterns of binding to leucocytes 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 HLDA8 website (www.hlda8.org) which will be replaced by a comprehensive web-based database providing links to protein and gene web-based databases (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, haematologists, 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 catalysed the development of an industry providing reagents for research, diagnosis and now therapy (see review by Boumsell, 1996²).

However, as molecular biological techniques grew in power, new molecules present on leucocytes were increasingly identified via gene cloning. Antibodies began to be made after the 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 four-year cycle, with the major aim of allocating CD numbers to well-characterised molecules against which good quality antibodies are available. However there has been increasing debate, within as well as outside the HLDA organisation, 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 summarised at the end of this article.

Methodology: the 8th HLDA Workshop

The 8th HLDA Workshop adopted the aims and methodology established at previous Workshops (since the changes detailed at the end of this article were not agreed 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. (4) To provide a

forum for discussing and developing scientific understanding and practical applications of leucocyte differentiation molecules, including their ligands.

Table 1 lists the 95 molecules that were assigned CD numbers as a consequence of the recent HLDA8 meeting. The antibodies validated against these “new” CD molecules, as well as newly validated antibodies against pre-existing 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 (Spiegelhalter and Gilks, 1987³; Gilks and Shaw, 1995⁴; Hallam et al., 1997⁵, Salganik et al., 2005⁶). 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 Non-Lineage 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 “re-discovering” 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.hlada8.org).

Results

The newly assigned CD numbers are shown in Table 1

Discussion: The future of HLDA

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 leucocyte surfaces (Zola & Swart, 2003⁷), 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 the following decisions:

1: Name change: The acronym “HLDA” will be succeeded by “HCDM” (for “Human Cell Differentiation Molecules”). The reasoning behind this name change is:

- a) To indicate a break with tradition, while retaining the letters “CD”.
- b) 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).
- c) To extend the focus from leucocytes to other cell types, recognising that leucocytes 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 non-haematopoietic human cell types.

- d) To broaden the scope from cell surface molecules to any molecule whose expression reflects differentiation. This would recognise the growing value of intracellular molecules, particularly in immunohistological studies of tissue sections, as markers of differentiation.

2: Nomenclature role: The HLDA Council is a sub-committee of the IUIS Nomenclature Committee, charged with responsibility for generating a consensus nomenclature for leucocyte 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 HUGO Gene Nomenclature Committee. CD numbers will not be allocated as routine practice to all leucocyte surface molecules recognised by newly available antibodies. CD numbers will continue to be allocated to molecules *where this appears likely to facilitate communication*.

3: 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 mis-assigned 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 – i.e. 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 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 recognised 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.

4: 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 which 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, haematology 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 organisation. 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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Table1 List of new CD assignments

NEW CD	MOLECULE	GENE ID*	GENE NAME
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	N/A
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 β 3 subunit	483	ATP1B3
CD299	DCSIGN-related	10332	CD209L
CD300a	CMRF35H	11314	N/A
CD300c	CMRF35A	10871	N/A
CD300e	CMRF35L1	N/A	N/A
CD301	MGL, CLECSF14	10462	CLECSF14

CD302	DCL1	9936	N/A
CD303	BDCA2	170482	CLECSF7
CD304	BDCA4, Neuropilin 1	8829	NRP1
CD305	LAIR1	3903	LAIR1
CD306	LAIR2	3904	LAIR2
CD307	IRTA2	83416	N/A
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	N/A
CD319	CRACC	57823	SLAMF7
CD320	8D6A	51293	N/A
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

* Gene ID refers to the Entrez Gene accession number.
<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene>