Nomenclature of CD molecules from the Tenth Human Leucocyte Differentiation Antigen Workshop

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**EDITORIAL**

Kohler and Milstein’s description of monoclonal antibodies (mAbs) in 1975 revolutionised immunology. With the advent of hybridoma technology, immunologists started to produce an ever increasing number of mAbs directed against leukocyte cell-surface molecules. Inevitably, several mAbs produced by different laboratories and given independent names recognised the same molecule. The First International Human Leucocyte Differentiation Antigen (HLDA) Workshop was organised in the early 1980s, and aimed to identify groups of mAbs reacting with a common human cell-surface antigen and to agree a nomenclature that would facilitate better communication amongst the scientific community. The outcomes of the Workshops are the ‘CDs’, an abbreviation for the non-descriptive ‘cluster of differentiation’ number. The CD number is assigned to a group or cluster of mAbs that recognise a molecule expressed on the surface of human leukocytes and other cells relevant to the immune system. The nomenclature has been universally adopted by the scientific community, officially approved by the International Union of Immunological Societies and sanctioned by WHO. Its usage has now evolved and is also commonly used to name the molecules themselves. However, by adding either mAb or molecule/protein it should be made clear whether one means the CDxx mAb or CDxx molecule.

Ten HLDA Workshops have been organised to date, with the most recent one held in 2014 in conjunction with the Australasian Society of Immunology in Wollongong, Australia. HLDA Workshops provide a unique forum based on an international exchange and blind evaluation of mAbs. Despite technological advances, especially in molecular biology, the basic protocol to establish a new CD remains essentially the same. The initial step consists in establishing a panel of mAbs submitted by numerous contributing academic laboratories and companies to an organising laboratory. This central laboratory distributes aliquots of each mAb among participating laboratories that perform specific studies with the blinded panel. Studies are primarily multi-parameter flow cytometry, but include immunohistochemistry and biochemistry. This allows for the testing of mAb-reactivity with multiple cell types including transfectants and healthy and clinical samples. The data are collected by the organising laboratory and analysed by using a hierarchical clustering algorithm. This kind of expression analysis is only possible in the context of a combined effort by a large group of laboratories.

Currently, the designation of a new CD requires at least two independent mAbs, submitted to the workshop that recognise the same molecule and have an identical pattern of reactivity. Proof of specific reactivity by using immunobiochemistry (immunoprecipitation, western blotting) and/or transfected cells is mandatory. Such mAbs have to specifically recognise the target protein on transfected cells and, importantly, the endogenous protein on primary cells. Cross reactivity between molecules is assessed and clarified. The collected data are presented at the HLDA Conferences and reviewed formally by the Human Cell Differentiation Molecule (HCDM) council, for mAbs that meet the requirements for a CD. A database with mAbs that have been approved by the HCDM can be found at www.hcdm.org.

The HLDA process provides an increasingly important function in independent validation of mAbs to improve scientific reproducibility. While mAbs have been produced by both academic groups and biotech companies to many molecules, a large number of these remain poorly validated. The use of non-properly validated antibodies has resulted in wastage of time and resources, destruction of research projects and generation of false results that have contaminated the scientific literature. The scientific community is increasingly aware of this serious problem, and the need to ensure scientific results are reproducible. The HLDA Workshop protocol reports on the positive validation of the submitted mAbs and is an invaluable tool for safeguarding and improving our knowledge of CD molecules and their function.

The data generated by the Workshops have led to the formal designation of 408 molecules (some of which are grouped within a CD) that has been reviewed recently. The HLDA10 Workshop tested a panel of 84 mAb provided by 12 groups, including commercial companies. The full list of mAbs tested is presented on the HCDM website (www.hcdm.org). Fifteen international groups contributed in testing the panel. This resulted in newly designated CD markers. The collection of reports published in the Clinical and Translational Immunology reviews and presents the work performed by HLDA10.

**NEW CD MOLECULES**

**CD365 (HAVCR1, TIM-1)**

The two antibodies 10-14 (clone FAB1750P) and 10-67 (clone 1D12) recognising TIM-1 (HAVCR1) were tested in the workshop. TIM-1 is a single pass type-1 membrane glycoprotein that is a member of the Ig superfamily. The antibodies bind to transfectants expressing TIM-1, but show little reactivity to fresh healthy blood cells.

**CD366 (HAVCR2, TIM-3)**

Transfectants verified that 10-24 (Clone 344823) bound to TIM-3. Like TIM-1, TIM-3 is a single pass type-1 membrane glycoprotein.
Two antibodies to TIM-3, 10-24 and 10-75 (clone F38-2E2), were used in the extended testing. They bound weakly to myeloid cell lines NB4, THP-1 and U937, as well as to monocytes and CD1c DC. They showed clear reactivity to the blast population in three AML samples used in the extended testing. They bound weakly to myeloid cell lines NB4, THP-1 and U937, as well as to monocytes and CD1c DC. They showed clear reactivity to the blast population in three AML samples.

CD367 (CLEC4A, DCIR)
We demonstrated binding of two antibodies 10-13 (clone 216110) and 10-71 (clone 111F8.04) to transient transfectants expressing DCIR. These antibodies showed strong binding to myeloid populations within PBMC. These antibodies recognise a type II transmembrane protein that is a member of the C-type lectin family.

CD368 (CLEC4D)
We demonstrated binding of two antibodies 10-21 (clone 413512) and 10-78 (clone 9B9) to transient transfectants expressing CLEC4D. These antibodies showed strong binding to myeloid populations within PBMC. These antibodies recognise a type II transmembrane protein that is a member of the C-type lectin family.

CD369 (CLEC7A)
Three mAbs 10-01 (clone GE2), 10-35 (clone 259931) and 10-79 (clone 15E2) to CLEC7A were tested. They demonstrated distinct but weak binding to transfectants and all bound well to monocyte and myeloid DC from PBMC. CLEC7A has also been referred to as Dectin-1, beta-glucan receptor and C-type lectin superfamily member 12 among other names.

CD370 (CLEC9A)
Three different mAbs, 10-02 (10-65, both clone 8F9), 10-09 (clone 9A11) and 10-45 (clone 683409), with reactivity to CLEC9A were submitted and tested in the Workshop studies. All three clones bound to transfectants expressing CLEC9A cDNA. There was only very weak reactivity to any cell line, however, all clones bound to the rare CD141+ DC population in peripheral blood. This was consistent with the reports in the literature. CLEC9A, also known as DNGR, is a type II transmembrane glycoprotein member of the C-type lectin family that functions as an endocytic receptor, particularly for the uptake and processing of dead cells through its ability to bind filamentous actin.

CD371 (CLEC12A)
Three antibodies 10-17 (clone HB3), 10-51 (clone 687317) and 10-73 (clone 50C1) to CLEC12A were tested in the HLDA10. Two of these mAbs, 10-51 and 10-73, were able to bind transfectants and showed strong binding to the myeloid populations of PBMC and to the three AML samples that were tested in the Workshop. CLEC12A, also known as MICL and CLI, is a type II transmembrane protein.

CONCLUDING REMARKS
The HLDA Workshops exemplify the benefits of a remarkably sustained, collective and collaborative international scientific effort. Future Workshops should continue to promote the exchange of reagents between academic groups and industry. They will boost the characterisation of high quality mAbs for all cell-surface molecules and provide an opportunity to check their integrity. The Workshops increase our understanding of leukocyte biology and pathology, and increasingly facilitate the identification of new disease biomarkers and therapeutic targets.
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