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CLUSTER OF DIFFERENTIATION ANTIGENS: A DIAGNOSTIC TOOL IN IMMUNOHAEMATOLOGY

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Abstract: *Cluster of differentiation antigens also known as cluster of differentiation marker[CD marker] are specific type of molecules found on the surface of cell membrane that help differentiate one cell type from another. This is used in identification and characterization of leucocyte cells and other cells that are important to the immune system. Due to the proliferation of many monoclonal antibodies discovered from different laboratories around the world. Their arose the need for harmonization of these monoclonal antibodies used by assigning CD antigens number when two monoclonal antibodies react with a surface antigen. Cluster of differentiation antigens naming system was created after the first international workshop held in paris in 1987, using different monoclonal antibodies, later other international workshop was held in different countries in subsequent years leading to about 391 CD markers. In immuno haematology, diseases such as leukemia, lymphoma, HIV and trasfusion transmitted diseaeses, can be diagnosed and therapy monitored using CD antigens with the method of flow sytometry. Hence much review has to be done to explore its diagnostic and therapeutic use especially during this covid-19 pandemic.*

Keywords: Cluster of Differentiation Antigens, Diagnostic Tool In Immunohaematology, differentiation marker

Background

Cluster of differentiation antigens also know as cluster of differentiation marker (CD Maker) are specific types of molecules found on the surface of cells membrane that help differentiate one cell type from another (1). Start it can further be defined as, cell surface markers used in the identification and characterization of leukocytes and other cells important for the immune system. (2). Other scholars defined it as a procedure used for identification and investigation of cell surface molecules providing targets for immunotying of cells.

History/Origin of Cluster of Differentiation Antigens

The CD antigens standard system of naming was proposed and established in the 1st international workshop and conference in 1982 (4) on human leukocyte differentiation antigens (HLDA) held in paris. The purpose of the naming system was for the classification and identification of many monoclonal antibodies (Abs) invented by different laboratories around the world against antigen binding sites found on the surface membrane of white blood cells. It's use has gained ground to many other cell types and now we have about 371 CD unique clusters & sub clusters (3)(2)(5).

The intending surface molecule is given a CD number, once, two specific monoclonal antibodies (mAb) binds to the antigen binding site. If the molecule has not been well characterized, or has only one mAb, it is usually given the provisional indicator "w" e.g "CD w 184". Therefore mAbs that have similar pattern of reactivity with various tissues or cell types were assigned to a cluster group (2, 4).

An antigen that is recognized by a cluster of antibodies can be assigned a cluster of differentiation number or CD number. Up to ten (10) international workshops

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have been held to compare the activities, of these monoclonal antibodies. The last conference held in Kobe Japan, November 1996, compiling the data originating from testing hundreds (100) of different mAb, this led to the classification of about 371 CD antigens. (5)(2).

Justification: There has been energy and reamergsity diseases that is troubly humanity, monoclonal antibodies has been of great importance both in diagnosis, treatment it disease monitoring.

Clusters of differentiation antigen which had been denied from surface moleeale reaction their specified monoclonal Abs had seen used could be app in immunohamalign especially in type down heamatological malignances. Immurophgrotypically. Therefore the aim of this resend relier is conduct extensity litenfie review CD makes to provide cabal information.

The aim of this article is to provide elaborate information on CD antigens as an important tool in immuno-haematology, emerging and reemerging diseases, and other metabolic disorders. 2004 is a long time there should be a recent international workshop on CD markers to update the old ones.

TYPES OF CLUSTER OF DIFFERENTIATION ANTIGENS

There are various types of Cluster of differentiation Antigens and their corresponding cells.

Type of cell	CD markers
Stem cells	CD34+.CD31-, CD117
All leukocyte groups	CD45+
Granulocyte	CD45+, CD11b, CD15+, CD24+, CD114+, CD182+([16])
Monocyte	CD4, CD45+, CD14+, CD114+, CD11a, CD11b, CD91+, [(16)] CD16+[(17)]
T lymphocyte	CD45+, CD3+
T helper cell	CD45+, CD3+, CD4+
T regulatory cell	CD4, CD25, FOXP3 (a transcription factor)
Cytotoxic T cell	CD45+,CD3+,CD8+
B lymphocyte	CD45+, CD19+, CD20+, CD24+, CD38, CD22
Thrombocyte	GD45+, CD61 +
Natural Killer cell	CD16+, CD56+, CD3-, CD31, CD30, CD38

Human Leukocyte Differentiation Antigen Workshops

Since 1982 there have been the ten human leukocyte differentiation Antigen Workshops

Workshop	City	Year	CDs assigned	Reference
1.	Paris	1982	1-15	Bernard etal 1984.
2.	Boston	1984	16-26	
3.	Oxford	1986	27-45	Reinherz ELet al 1985.
4.	Vienna	1989	46-78	Knapp W;etal1989.
5.	Boston	1993	79-130	Knapp et al 1989.
6.	Kobe	1996	131-166	Schlossman SF,1995.
7.	Harrogate	2000	167-247	Kishimoto,T.,etal 1997.
8.	Adelaide	2004	248-339	Mason,D.,2002.

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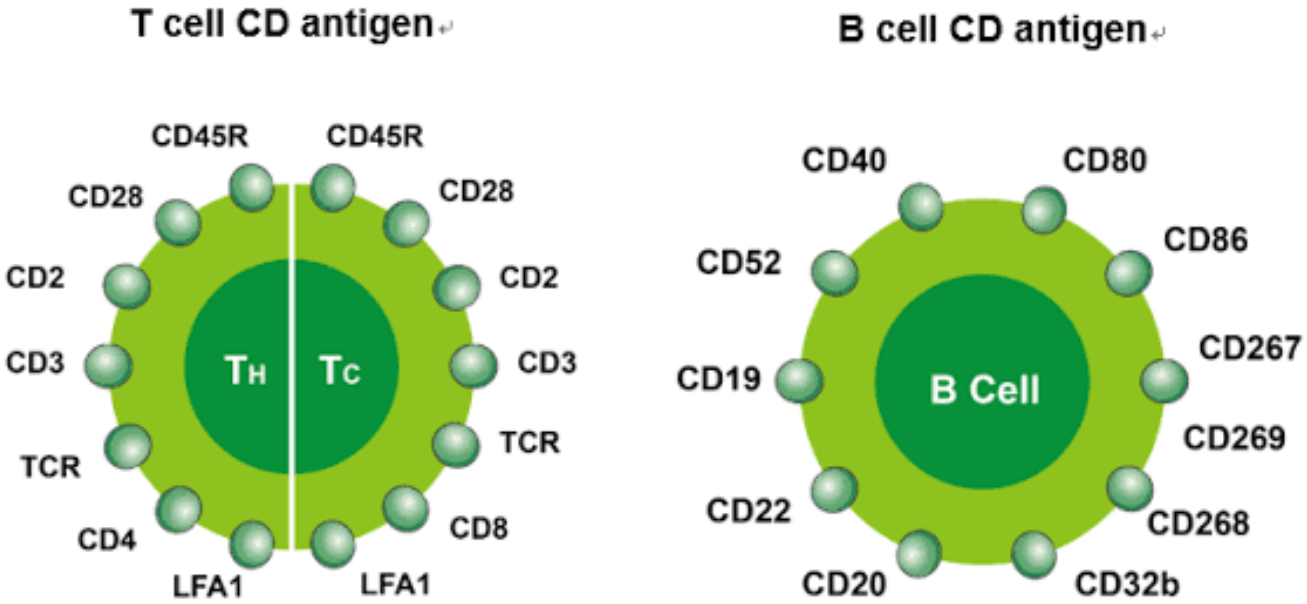
9.	Barcelona	2010	340-364	
10.	Wollongong	2014	365-371	

(Zokaer 2005)



THE MECHANISM OF ACTION OF CLUSTER OF DIFFERENTIATION ANTIGENS OCCURS

in numerous ways they act as receptors or ligands (i.e. a molecule that activates a receptor) important to the cell. A signal cascade is usually initiated altering the behaviour of the cell. Even though some do not play a role in cell signaling, they perform other functions such as cell adhesion, cell activation and cell inhibition. Throughout the active life of the cells they interact with an adhesion complex. These complexes interact with the cellular substrate or extracellular matrixes and with one another (6).

These complex cellular structures involve many proteins, such as CD antigens. These junctions are mediated by either trans-membrane cell adhesion molecules or adhesion receptors. Cell adhesions are mediated by either trans-membrane cell adhesion molecules or adhesion receptor. The adaptive immune system or specific immune response consists of antibody responses and cell-mediated responses which are carried out by different lymphocyte cells, mainly B cells and T cells respectively. (7).

Some CD Antigens are cells surface protein and act as receptors (2, 4). The CD antigens synergisation is basic to cell signaling. When such CD antigens trigger off its receptors, the signal is carried into the cells usually by means of a second messenger (2) CD 40, a ligand for the B cells is a solution/pointer to the activation of the cells' antigen. B7 protein is present on the antigen cells surface of macrophages & dendritic cells and it synergizes with CD28 receptor on T cell surface. We have two types B7 proteins: B7-1/CD80 and B7-21 C8 (Woolfson et al; (2006).

Function of CD Antigens.

(1) Immunophenotyping

The CD system is commonly used as cell markers in immunophenotyping, allowing cells to be defined based on what molecules are present on their surface. These markers are often used to associate cells with certain immune functions. While using one CD molecule to define populations is uncommon (through a few examples exist), combining markers has allowed for

cell types with very specific definitions within the immune system to be identified.

CD molecules are utilized in cell sorting using various methods including flow cytometry. It is used for cell signaling and cell adhesion (2). The frequently two commonly used CD molecules are CD4 and CD8, which are usually used as markers for helper and cytotoxic T cells, respectively. These markers molecules are defined in combination with CD3+. As some other leukocytes also express this CD markers molecule, some macrophages express low levels of CD4; high levels of CD3 are expressed in dendritic cells, and also express high levels of CD8). Human immunodeficiency virus (HIV) binds CD4 and a chemokine receptor on the surface of a T helper cell to gain entry into the cell. The numbers of CD4 and CD8 T cells in blood are used to monitor the progression of the disease. This can equally be applied to covid-19 pandemic(16).

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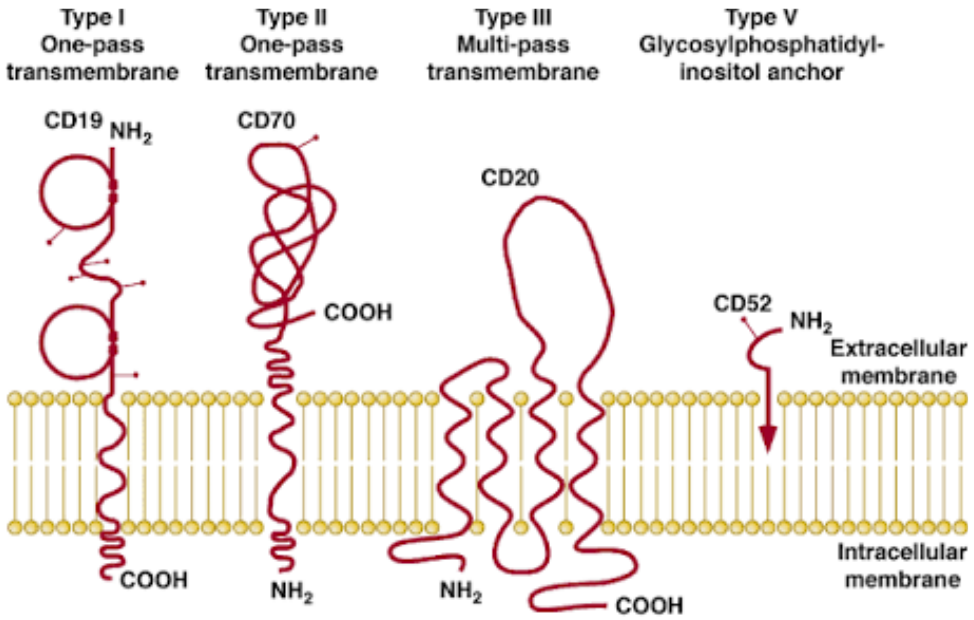
Classification of cluster of differentiation antigens.
Membrane antigens (CD Antigens) are classified into different groups, depending on how they orient or anchor themselves to the plasma membrane (14).



This figure depicts the major different types of surface proteins with respect to how they integrate into the membrane bilayer. The types of membrane protein are indicated at the top of the figure. The straight lines attached to the open circles represent the lipid bilayer. The dark black lines represent the polypeptide backbones, and the thin black pegs extending from the polypeptide backbone represent carbohydrates. At the far left is CD19, a type I transmembrane protein that passes through the membrane once and has the C-terminus (COOH) in the cytoplasm and N-terminus (NH_2) outside the cell. To the immediate right is CD70, a type II single-pass transmembrane protein with the N-terminus inside the cell. To the right of this is CD20, a type III multispan protein that also is a tetraspan molecule in that it traverses the lipid bilayer four times. The tetraspan proteins have both the N-terminus and C-terminus in the cytoplasm. To the far right is CD52, a glycosyl-phosphatidylinositol (GPI)-anchored protein. The labels at the far right indicate the extracellular and intracellular membranes.

TYPE I TRANSMEMBRANE PROTEINS (I)

Type I transmembrane molecules have their COOH-termini in the cytoplasm and their NH_2-termini outside the cell. Each of these molecules generally has a signal sequence at the NH_2-terminus that is cleaved off after the molecule passes into the endoplasmic reticulum. Afterwards it may be glycosylated in the Golgi apparatus (if it contains glycosylation sites) and then expressed on the cell surface. These proteins commonly serve as cell surface receptors and/or ligands. Many belong to the immunoglobulin superfamily. Functions of B lymphocytes and Plasma Cells, and functions of T Lymphocytes). (13, 14).

Each type 1 protein generally has a transmembrane domain of approximately 25 hydrophobia amino acid residues followed by a cluster of basic amino acids that bind the protein to phospholipid head groups inside the surface membrane bilayer The transmembrane domain does not contain any charged amino acid residues, such

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as Arg, Asn, Asp, Glu, Gin, His, or Lys, except when it associates with the transmembrane domain of another cell surface protein to form a multimeric complex. An example of this is the multimeric complex formed by the CD3 proteins and the two chains of the T-cell receptor for antigen (13)(14).

TYPE II TRANSMEMBRANE PROTEINS (II)

Type II transmembrane proteins have opposite orientation to that of type I transmembrane proteins. The NH_2-terminus is located inside the cell, and the COOH-terminus is extracellular. These proteins often have uncleaved signal sequences for transmembrane domains, allowing for their cleavage and release from the cell surface. As such, these proteins may double as cell surface antigens and plasma proteins, each often having a physiologic effect on cells bearing the respective ligand (13)(14).

TYPE III TRANSMEMBRANE PROTEINS (III)

Type III transmembrane proteins cross the plasma membrane more than once. Some pass through the bilayer as many as 12 times, such as the multidrug resistance transporter protein, MDR1. Because they cross the membrane multiple times, these molecules can form channels that often are used to transport ions or small molecules through the lipid bilayer. An important subgroup of type III transmembrane proteins that commonly are found on leukocytes is the tetra-span family. These proteins each pass through the surface bilayer 4 times and have both their COOH-termini and NH_2-termini inside the cell. An example is CD20, a molecule that is postulated to form a calcium channel for B lymphocytes that is required for B-cell activation.

TYPE IV TRANSMEMBRANE PROTEIN (IV)

Type IV proteins can be distinguished from type II proteins by the presence of a water-filled transmembrane channel. None of these membrane organization current CD antigens have such a membrane organization.

TYPE V GLYCOSYL-PHOSPHATIDYLINOSITOL-ANCHORED PROTEINS

Type V proteins use lipid to attach themselves to the plasma membrane. The most common attachment for extracellular proteins in this category is the glycosyl-phosphatidylinositol (GPI) anchor. The GPI anchor can be cleaved by the bacterial enzyme, phosphatidylinositol phospholipase C (PI-PLC). Release of an antigen from within the cell surface by treatment with PI-PLC often is used to verify that the surface protein has a GPI anchor. However, this criterion is not absolute, as some GPI-anchored proteins may be resistant to PI-PLC(12)(14).

Newly synthesized proteins destined to receive a GPI anchor each contain a secretion signal sequence at the NH_2-terminus and another signal sequence at the COOH-terminus. The latter directs cleavage and subsequent appendage of a GPI anchor soon after the molecule's biosynthesis and extrusion into the endoplasmic reticulum. This biosynthetic pathway is defective in paroxysmal nocturnal hemoglobinuria (PNH).

The site of attachment for the GPI generally precedes a hydrophobic domain of 7 to 20 amino acids that sometimes may double as an actual transmembrane domain. In this case, the molecule may exist as either of two isoforms, one attached to the membrane via a GPI anchor and another as a type I transmembrane protein. Because GPI-anchored proteins associate specifically with sphingomyelin lipids, follow a different path of transport to the cell surface than type I transmembrane proteins, are excluded from coated pits, and are not able to associate directly with intracellular proteins, a GPI isoform of a given surface protein usually has a physiology that is distinct from that of its respective type I transmembrane isoform (15).

TISSUE DISTRIBUTION OF MEMBRANE ANTIGENS

The tissue distribution for each CD antigen listed summarizes the work of many laboratories. For most CD antigens, however, a comprehensive analysis of the full

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gamut of different tissues has not been performed. Therefore, failure to list a cell type in this table for a particular CD antigen does not necessarily mean that cell type does not express that antigen. A complete review of the tissue distributions of the CD antigens is reviewed in the summary books published after each workshop. References to these books are implied, but not necessarily cited, for each CD antigen listed (14)(15).

Some of surface antigens are useful for delineating the cell lineage of leukocytes. Unique assignment of a surface antigen to a particular lineage is best when the antigen is related to a unique functional property of a given cell type. The CDS surface antigens form part of the T-cell receptor complex or antigen. As such, CDS is expressed exclusively by mature lymphocytes of the T cell lineage. In a similar vein, surface immunoglobin (sIg) is a B-cell lineage specific marker. In addition, CD20 is another antigen found exclusively on lymphocytes of the B-cell lineage (15).

Most CD antigens, however, are expressed at varying levels by many different cell types. Rather than the exclusive expression of a single CD antigen with a particular cell type, it is the peculiar constellation of surface antigens expressed by a given cell that helps assign it to a particular lineage or sublineage of cells. Increasingly, the resolution of many important cells subpopulations requires two or more colour multi-parameter flow cytometric analyses.

RELEVANCE OF CLUSTER OF DIFFERENTIATION MARKERS IN IMMUNOHAEMATOLOGY AND COVID 19 PANDEMIC.

Coronovirus-2-disease otherwise known as covid-19 pandemic caused by severe acute respiratory syndrome coronavirus2(SARS-CoV-2) is a global health problem that has killed many and destroyed global economy(16). This disease originated from Wuhan-China in 2019 “where a cluster of viral pneumonia cases was first detected, many in connection with the Huanan Seafood Wholesale Market. China reported this outbreak to the WHO on December 31, 2019 and soon after identified the causative pathogen as a betacoronavirus with high sequence homology to bat coronaviruses (CoVs) using angiotensin-converting enzyme 2 (ACE2) receptor as the dominant mechanism of cell entry (16)(17).

Different researchers have proposed different mechanisms of action of this virus in human body but, Nie *et al* 2020 have confirmed that leucocytes are affected during corona-virus infection mainly the lymphocytes, Lymphopenia has been associated with this disease. The extent of Lymphopenia-most striking for CD8 T cells in patients admitted to the intensive care unit (ICU)-seemingly correlates with covid-19-associated disease severity, and mortality (17), (18), (16), Wang *et al*, 2020f; Zeng *et al*, 2020). Patients with mild symptoms, however, typically present with normal or slightly low helper T cell counts (16), Thevarajan *et al*, 2020). CD19,CD20,and CD22 can be used the monitor the Bcell antibody production in immunosuppred diseases as in covid–19 using the method of flow cytometry.CD45,CD4, are also important in typing and monitoring haematological malignancies, such as leukemia ,lymphoma.

Some of the CD Makers found in different leukemias and lymphomas are as shown bellow.

Acute basophilic leukemia myeloid markers present	Immunophenotpying	CD 9, CD 13, CD 33
Acute leukemia of ambiguous Lineage(Biphenotyic leukemia)	Immunophenotpying	a. CD 13, CD 14, CD 15, and/or CD Positive with myeloid line b. CD 19, CD 10, CD 7, and/or CD2, positive with lymphoid line
Acute megakaryoblastic leukemia	Immunophenotpying	a. CD 41 and/or CD 61 positive b. CD 42 may be positive if the cell is more

		mature c. CD 34 and CD 45 often negative.
Acute myelogeneous leukemia minimal, differentiate	Immunophenotpying	a. >20% blast react with mseloid antigens (CD 33, CD 34, CD 117 and HLA-DR). b. Negative for lymphoid antigen (CD 3, CD5, CD 10, CD 19, CD 20, CD 22)

Acute myelogeneous leukemia	Immunophenotpying	CD 13, CD 33, CD 117 & CD 15
Chronic mgelocytic leukemia		CD 7
Acute lymphoblastic leukemia	Immunophenotyping	CD 19, CD 20
Chronic lymphocytic leukemia	Immunophenotyping	CD 5, CD 19, CD 23 & CD 7
Barkiti's lymphoblastic	Immunophenotyping	CD 19, CD 20
Hodgkin lymphoma	Immunophenotyping	CD 20, CD 30, CD 15
Non-Hodgkins lymphoma	Immunophenotyping	CD 19, CD 20 & CD 45.

CONCLUSION

Cluster of Differentiation Antigens are of immense advantage to long-term international collaboration across different countries involved in human leucocyte antigens and monoclonal antibody identification which helps to facilitate diagnoses of haematological malignancies such as lymphomas and leukemias, new emerging diseases, and its use for therapeutic agents and even in monitoring the prognosis of diseases such as HIV, transfusion transmitted diseases and probably Corona Virus- disease pandemic. More CD markers should be invented for coronovirus 2 pandemic.

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