

## CD38 MOLECULE-A MULTILINEAGE GLYCOPROTEIN AND ITS UNIQUE EXPRESSION ON PLASMA CELLS

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### ABSTRACT

A hybridoma clone designated 6G5 has been selected by fusion of mouse myeloma cell line Ag. 8653 with spleen cells from mice immunized with human peripheral blood mononuclear cells (PBMC). The antibody produced by this clone was found to be strongly reactive with four human B-cell lines in the conventional immunological assays. Despite the fact that expression of most B cell-associated markers are lost upon differentiation of B-cells to plasma cells, the expression of the 6G5 reactive molecule remains unchanged. The lack of reactivities of this MAb for mature T-cells, and monocytic cell lines indicates that this MAb recognizes a B cell-associated marker. Western blot analysis indicated that the 6G5 MAb detected a single band with molecular weight of 41KDa from cell lysates of two human B-cell lines, DAUDI and NALM6. Comparison of data obtained for 6G5 MAb with those of the MAb known as OKT10 indicated that both MAbs may have reacted with the same molecule.

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### INTRODUCTION

Leukocyte-associated glycoproteins are generally categorized as lineage-restricted markers, leukocyte common markers and activation markers. Lineage-restricted markers are specific glycoproteins expressed on each lineage and represent the entity of every lineage derived from hemopoietic hierarchy. The availability of monoclonal antibodies to these specific markers, the preparation of single cell cultures, and determination of the changes in leukocyte subsets in health and disease by employing the new and powerful technology of flow cytometry has become a technical reality. A question yet to be answered is what role these markers play in lymphocyte physiology. In general, these could well be considered as growth factor receptors, or a means of cell locomotion and adhesion. This is defined partly by the biochemical properties of these molecules, namely a 35 KDa glycoprotein known as CD20, a well-studied B-lymphocyte restricted marker. CD20 is expressed on the pre-B cell lineage to the mature activated stage of B

lymphocytes and is not present on the surface of plasma cells. The biochemical characteristics and membrane orientation of this molecule with its large transmembrane portion all concern with a possible function in signal transduction and activation.<sup>1,2</sup> The leukocyte common antigen always refers to those glycoproteins shared by most bone marrow-derived cells. This is exemplified by the CD45 (T200, B220) molecule and its different isoforms which differ in their tissue distribution, carbohydrate content and protein sequences.<sup>3,4</sup> Activation molecules are referred to the membrane-associated glycoproteins which are absent on resting leukocytes but their expression is largely dependent on cognate interaction of lymphocytes with antigen-presenting cells, or the existence of cytokines. These are exemplified by the up-regulation of interleukin-2 receptors (IL-2R) on activated T cells<sup>5</sup> and short transient expression of CD23 on B lymphocytes.<sup>6</sup> It is assumed that the transient expression of an activation marker within the specific stage of cell cycle is associated with a defined function.<sup>6,8</sup> A few leukocyte markers have been reported to express the

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properties of three types of leukocyte surface antigens. Of these, a 42 KDa multilineage glycoprotein clustered as CD38 antigen which is transiently expressed on early lymphocyte precursors and more consistently on plasma cells is worth mentioning. This is unique among most B-cell associated molecules whose expression are lost at the plasma cell stage. CD38 expression on thymocytes and activated T-cells is well documented.<sup>9</sup> Recent study has shown that MAb to CD38 is able to induce activation of other molecules such as HLA class-II and IL-2R.<sup>11</sup> Transmembrane signaling through the cross-linking of CD38 molecules has also been reported.<sup>11</sup> In this study we report the specificity of a monoclonal antibody recently produced in our laboratory. It is directed against a 41 KDa membrane-associated molecule expressed on B-cells, plasma cells and thymocytes. Our data indicate that this MAb (designated as 6G5) has a pattern of reactivity similar to those MAb already produced against CD38 molecules.

### MATERIALS AND METHODS

#### Medium and reagents

PRMI-1640 medium, heat inactivated fetal calf serum (FCS), penicillin and streptomycin were all obtained from Gibco, Scotland. Peroxidase and fluorescent conjugated goat anti-mouse Ig, hypoxanthine, thymidine, aminopterin, thioguanine and polyethylene glycol were purchased from Sigma, USA.

#### Cells and cell lines

Peripheral blood mononuclear cells (PBMC) were obtained from healthy volunteers. Thymocytes were prepared by teasing of thymus tissues obtained from patients undergoing open heart surgery. Bone marrow cells were obtained by routine bone biopsy. JURKAT and HPB-ALL (T cell lines) and U937 were kindly provided by Dr. R. Tiebout of the Netherlands Red Cross Blood Transfusion Center. The NALM6, NALM16 (pre-B cell line) and BJAB, DAUDI, RAMOS (Burkitt's lymphoma cell lines) were obtained by the courtesy of Dr. A. Hekman of the Netherlands Institute of Cancer, Amsterdam.

#### Production of monoclonal antibody and fusion protocol

6G5 MAb was produced as previously described.<sup>11</sup> Briefly, the BALB/c mice were immunized with mononuclear cells prepared from tonsils. Spleen cells from immunized mice were fused with Ag8.653 mouse myeloma cell line by the use of polyethylene glycol as fusogen. Growing hybridomas were detected 7-10 days postfusion and screened to obtain the desired monoclonal antibody producing clones.

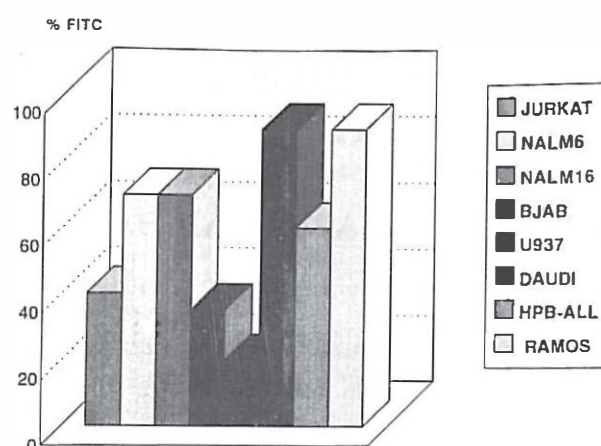


Fig. 1. Reactivity of 6G5 MAb with a panel of hemopoietic cell lines in an indirect immunofluorescent test. Results shown are the mean of four separate experiments. For details of the experiment and more information about the cell lines see Methods section.

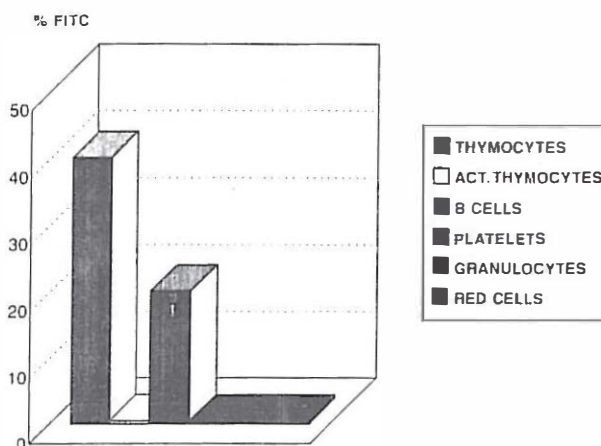


Fig. 2. Reactivity of 6G5 MAb for normal T and B lymphocytes prepared from thymus and tonsil tissues respectively. Results are the mean of four separate experiments.

#### Indirect fluorescent test (IFT) and immunoperoxidase staining

Reactivity of the 6G5 MAb for different cell lines were tested by IFT as described before.<sup>11</sup> Frozen section from tonsil and thymus tissues were stained by 6G5 MAb as described.<sup>12</sup> Briefly, the slides were gradually brought to room temperature and incubated with 6G5 MAb for 30 min. Slides were extensively washed with PBS. After washing, slides were incubated with conjugated goat anti-mouse peroxidase for a further 30 min at room temperature, followed by addition of diaminobenzidine tetrahydrochloride in PBS and a few drops of hydrogen peroxide as substrate solution.

#### Immunoblotting analysis

Determination of the membrane-associated marker



Fig. 3. Immunoperoxidase staining of a plasma cell from bone marrow biopsy by 6G5 MAb (magnifica

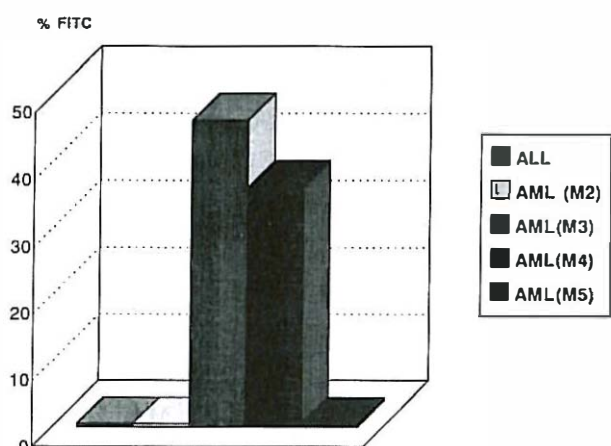
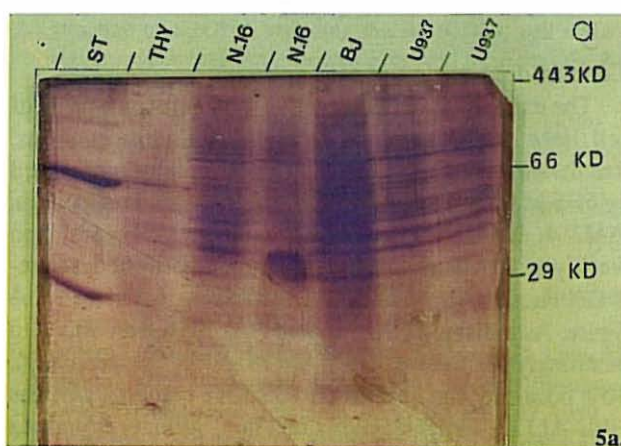


Fig. 4. Reactivity of cells from one case of acute lymphoblastic leukemia and four cases of acute myelogenous leukemia with 6G5 MAb in an indirect immunofluorescent test.

reactive with 6G5 was carried out as recently described.<sup>11</sup> BJAB and NALM16 cell lines were solubilized using a lysate buffer (PBS containing 0.05% triton X100, 20mM PMSF 10mM EDTA). The cell lysate was electrophoresed on 10% acrylamide gel under reducing and non-reducing conditions. Following electrophoresis the protein was transferred onto nitro-cellulose membrane using Novablot 2 (LKB-Pharmacia). In order to block any possible non-specific binding the nitro-cellulose papers were incubated in PBS-Tween containing 2% goat serum. Then the nitro-cellulose membranes were incubated with 6G5 MAb for two hours followed by an extensive washing and applying a peroxidase conjugated goat anti-mouse Ig as a probe. The nitrocellulose membranes were washed and developed using diaminobenzidine tetrahydrochloride as peroxidase substrate.



5a.

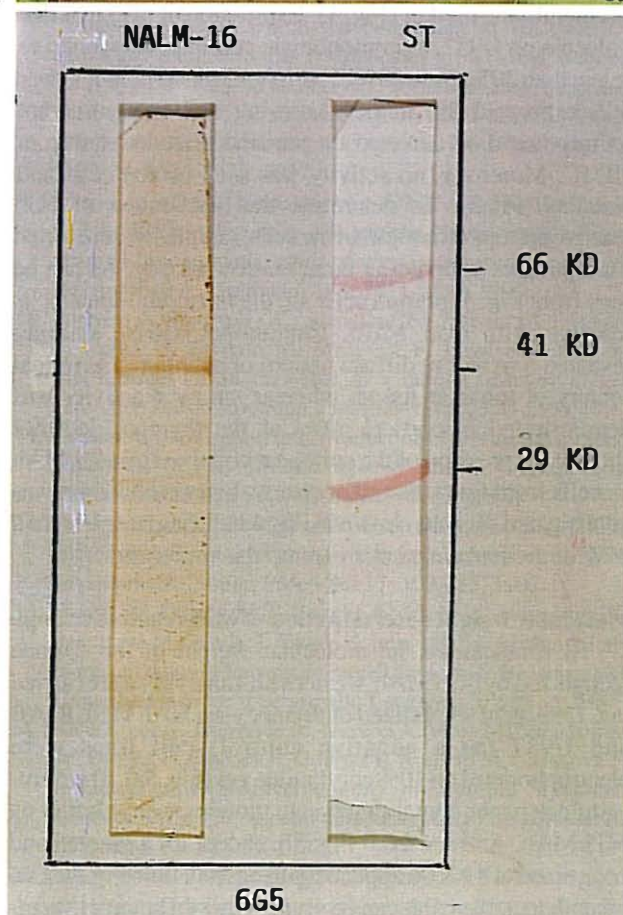


Fig. 5. Cell lysate prepared from thymocytes (THY), NALM16 (N-16), BJAB(BJ) and U937 cell lines were electrophoresed on a 10% polyacrylamide SDS gel under reducing conditions (a). Proteins in the gel were transferred on the nitrocellulose paper and blotted by 6G5 MAb ST (standard molecular weight markers).

## RESULTS

### Cellular distribution of 6G5 epitope

The expression of 6G5 reactive molecules on a panel of cell lines was determined by indirect fluorescent tests. Results shown in Fig. 1, indicate that the epitope recognized by 6G5 MAb was strongly detected on B-cell lines such as DAUDI, RAMOS (Burkitt's lymphoma cell lines) and weakly on BJAB cell line. Reactivity of this antibody to pre-B cell lines (NALM6 and NALM16) is shown in the same figure. As indicated NALM6 and NALM16 were found to be strongly positive for 6G5 MAb (78 and 98% respectively). 40% positivity can be seen on JURKAT cell line, whereas HPB-ALL, a T-cell leukemia cell line, strongly expressed this membrane marker (Fig. 1). Expression of 6G5 reactive molecule on U937, a promonocytic cell line, was found to be less than 20%. Only 20% of peripheral blood mononuclear cells expressed this marker knowing that no fluorescent activity could be detected on purified granulocytes from PBMC. Moreover, no activity was seen on red cells and platelets (Fig. 2). To determine the distribution of 6G5 reactive epitope on bone marrow cells, samples were selected from patients undergoing bone marrow biopsy. As can be seen from Fig. 3, plasma cells are the most prominent cells reactive with 6G5 MAb. Immunoperoxidase staining revealed a weak but diffuse pattern of staining in germinal centers of tonsillar tissue, whereas strong reactivity was demonstrated in cortical areas of the thymus (data not shown). Expression of the epitope recognized by 6G5 MAb on cells from four cases of acute myelogenous leukemia was investigated. Results shown in Fig. 4 indicated that less than 35% of the leukemic cells express this molecule.

### Molecular weight determination of 6G5 reactive epitope

To characterize the molecular weight of the epitope recognized by 6G5 MAb, western blot analyses were carried out. Detergent solubilized of thymocytes, NALM16, BJAB and U937 (as a negative control) cell lines were electrophoresed on 10% acrylamide gel (Fig. 5a). The nitrocellulose paper containing lysate proteins were blotted by 6G5 MAb. As seen from Fig. 5b, except for a single band comprised of 41 KDa appearing on nitro-cellulose paper, no other detectable band can be seen. When these experiments were repeated under reducing conditions, no changes were seen on the pattern of protein migration on the gel (data not shown). Similar experiments were repeated on cell lysate prepared from JURKAT and DAUDI cell lines which indicated a similar pattern of the molecular size of the 6G5 reactive epitope (data not shown).

## DISCUSSION

The hybridoma clone producing 6G5 MAb was selected

after fusion of a mouse myeloma cell line with mouse spleen cells immunized with purified mononuclear cells from tonsils. Cellular distribution of epitope recognized by 6G5 MAb indicated that this MAb did not react with a lineage restricted marker. Cortical thymocytes, a small fraction of peripheral blood mononuclear cells and plasma cells within the bone marrow expressed this molecule. Despite the weak expression of the molecule on a minor population of B-cells, two pre-B cell lines were strongly reactive with this MAb. Lack of reactivity of 6G5 MAb with U937 cell lines, granulocytes, eosinophils, platelets and red blood cells indicate that 6G5 probably recognizes a lymphocyte-associated marker. With respect to strong reactivity of this MAb with cortical areas of the thymus it was assumed that this MAb is directed against a specific and early thymocyte antigen. But reactivity of 6G5 MAb with DAUDI, REMOS (Burkitt's lymphoma B-cell lines) and NALM6 and NALM16 (pre-B cell lines) and most importantly, strong reactivity with plasma cells of bone marrow origin clearly revealed that both T-cells and B-cells in some stage of their development may express this receptor. Expression of 6G5 epitope on B-cells seems to be somehow unusual and also interesting; strong association in the early stages of B-cell ontogeny, lack of expression on mature B-cells, followed by new synthesis on B-blasts and end stage B-cell differentiation, the plasma cells. This epitope resembles the CD10 molecule in its pattern of expression on B-cells. When this unique profile was compared with known B-cell associated markers, a quite similar line identity can be seen with those specificities which have been reported for monoclonal antibodies clustered as CD38. The reactivity of 6G5 MAb for cortical thymocytes is still consistent with tissue distribution of the epitopes for MAbs in the CD38 cluster.<sup>9</sup> To confirm this identity, the molecular size of 6G5 reactive protein and the CD38 molecule have been compared. As indicated in the results section, immunoblotting analysis by 6G5 MAb detected a 41KDa band from the solubilized membrane of thymocytes, NALM16 and BJAB cell lines but not from U937, a monocytic cell line. This data was unchanged under reduced and non-reduced conditions, where no other band on the nitrocellulose membrane was detected. This is in agreement with the molecular size which has been reported for the CD38 molecule.<sup>10</sup> Stamenkovic and his colleague<sup>1</sup> first described a monoclonal antibody directed against human T-cells which reacted with a single band comprised of approximately 42 KDa prepared from a human Burkitt's lymphoma cell line (DAUDI). In the Third Workshop for Human Leukocyte Antigens those antibodies with this specificity were clustered as CD38. The CD38 molecule is a glycoprotein which is distinguished from MHC-class I by a protein core of 35 KDa which has been revealed by use of endoglycosidase F,<sup>2</sup> and it is now known as a B-cell associated marker in contrast to the initial entity of its representation as a T-cell marker. Expression of CD38 glycoprotein on greater

than 90% of plasma cells from myeloma patients has been reported.<sup>13-15</sup> It is interesting to note that 6G5 MAb was detected on plasma cells purified from bone marrow aspirates of four patients with multiple myeloma. The expression of this marker on neoplastic plasma cells has led certain investigators to use MAb against CD38 in their therapeutical approaches. For instance Stevenson et al. reported the use of a chimeric anti-CD38 antibody for the treatment of human myeloma.<sup>16</sup> Results of the clinical trial on this group indicated an effective antibody-dependent cellular cytotoxicity (ADCC) with this engineered MAb with no deleterious action on hemopoietic progenitor cells. More work is needed to evaluate the cytotoxic effect of 6G5 MAb on neoplastic plasma cells and also to compare the epitope recognized by 6G5 MAb with the previously reported MAbs against the CD38 molecule. Moreover, it would be particularly interesting to study the fate of this molecule in normal plasma cells and on myeloma cells; for instance, is the CD38 molecule modulated spontaneously or by cytokines such as IL-6. More important questions to be answered are to what extent the 6G5 reactive molecule or CD38 glycoprotein has a role to play in the process of myeloma cell adhesion and locomotion. This is an area in which we have just started to concentrate and do more work on. A preliminary work in our laboratory strongly supports a decisive role for the CD38 molecule in activation and induction of essential surface markers on cortical thymocytes (Ghaderi and Amirghofran, manuscript in preparation). What is the role of this molecule on lymphoid progenitors in general? Would it have (as mentioned before) a role in leading early lymphoid stem cells to localize in a suitable lymphoid microenvironment and skip a negative selection process? To evaluate such a possibility, the CD38 natural ligand and its cellular and tissue distribution need to be explored. This issue is currently undergoing investigation in our laboratory.

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