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Tools for cellular immunology and vaccine research the in the guinea pig: Monoclonal antibodies to cell surface antigens and cell lines

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Abstract

The use of monoclonal antibodies directed against membrane proteins of leukocytes has greatly contributed to our understanding of the function and development of the immune system. Meanwhile these reagents provide valuable tools in many fields of research, stretching far beyond immunology and hematology. For guinea pigs only a limited number of such reagents have been described, and the information about availability and specificity is scattered over many years and journals. We provide an overview on the monoclonal antibodies produced since the technique was applied first in this species, with a focus on those reagents which have been characterized in more detail, and which should still be available either commercially or directly from the labs that created them.

1. Introduction

The term “guinea pig” is used in the English language to both refer to the animal species with the Latin name *Cavia porcellus*, and to indicate that a certain animal or human is used as a test subject for medical treatments or other kinds of experimental procedures. Although the species stems from the South America and has been brought to the old world first in the 16th century, it has coined our understanding of an experimental animal in a way that we use the term “experimental animal” and “guinea pig” as synonyms. Historically the guinea pig was the most widely used animal model for infections in the 18th, 19th and early 20th century, and it largely contributed to the development of the concept of infectious diseases caused by pathogenic microorganisms. Although Robert Koch had first infected mice with tubercle bacilli, he used guinea pigs to establish bacteria as the etiologic agent that causes tuberculosis [1]. The remarkable similarities between humans and guinea pigs in many physiological traits have been outlined by several authors [2] and [3], and continue to give reason to use guinea pigs as the preferred model for various diseases and pathological conditions affecting humans, even if mouse or rat models for the same subject of research exist.

Regarding the range of available strains, including mutants, transgenic breeds generated by homologous recombination, and the availability of research tools such as recombinant cytokines, assay systems, and monoclonal antibodies (mAb) to detect and characterize cell lineages based the expression of surface antigens, the mouse is largely superior to every other experimental animal. Considering susceptibility to certain diseases, disease progression, and the immune response in infected mice however, alternative models using guinea pigs, rabbits, rats, and even non-mammalian species may turn out more suitable than mice to study a certain condition [4]. Most of these alternative models however suffer from a lack of genetically defined strains and absence of the research tools listed above. Therefore the mouse is still the most widely used animal for medical research, although for many diseases it is certainly not the best.

There is an abundance of reviews emphasizing the versatility of guinea pigs as research models in general [5], [6], [7], [8], [9], [10], [11], [12] and [13] or with reference to infectious diseases and vaccination [14], [15], [16], [17], [18], [19], [20], [21] and [22], with a strong focus on tuberculosis research [4], [23], [24], [25], [26], [27], [28], [29], [30], [31], [32], [33] and [34]. Guinea pigs represent the “gold standard” among small animal models to test the efficiency of new and established vaccines against tuberculosis [35], [36] and [37], and are useful for vaccination studies against other diseases [38], [39] and [40], but most authors agree that the limited availability of immunological reagents in this species strongly restricts its usefulness for modern experimental approaches. As a consequence
many studies on the immune response to these diseases have been conducted in animal models that have less similarity to humans, but have more immunologic reagents available. Although many research directions could benefit from an improved availability of monoclonal antibodies in the guinea pig, the highest demand is certainly in the area of immunology, vaccination and infectious diseases.

Several obstacles have affected the development of research reagents for guinea pigs. Most of them are associated with the higher costs of purchase and care of guinea pigs as compared to mice and rats. At the same time the limited number of inbred strains and the absence of transgenic and knockout animals have made guinea pigs less attractive to researchers even if the guinea pig was the more appropriate disease model.

This review aims to summarize efforts undertaken to generate mAb directed against guinea pig differentiation antigens. Most guinea pig research reagents were generated with the intention to address a specific problem and used solely for that purpose, few studies had the concept to generally improve the availability of experimental tools for research in guinea pig models. Therefore the available information is scattered over many diverse fields of research and published in various journals that are frequently of limited importance for colleagues working in different subjects such as veterinary animal science and animal models for human placentation or arthritis. Furthermore, frequent requests to our lab for mAb or recombinant proteins developed by others, indicate that the demand is high but the information on the source and availability of these reagents is limited, although most of the published work is accessible via the internet.

2. Phenotyping of leukocyte populations using differentiation antigens expressed on the cell surface

2.1. Cluster of differentiation antigens

Although mAb were used to define guinea pig differentiation antigens soon after utilization of the technology in human and mouse systems, it was restricted to a few specialized labs [41], [42], [43], [44], [45] and [46] and the guinea pig field remained rather neglected when the first workshops to define clusters of differentiation (CD) in the human system were held and the nomenclature became rapidly adopted to the mouse and rat systems. Meanwhile a number of mAb to guinea pig lymphoid and myeloid differentiation antigens have been reported, but only few of them have been assigned to the CD nomenclature and even less have been confirmed by molecular biology methods.

Some of the target antigens have meanwhile been cloned and the specificity of the mAb has been confirmed by recombinant expression (see Table 1 remarks). Ideally the identity of the antigen recognized by each mAb should be confirmed by cloning of the coding gene and recombinant expression into host cells which are otherwise not stained by antibodies directed against the respective antigen. Alternatively identification and thorough characterization of the bound protein can be performed. Some specificities have been assigned on the basis of potential inter species cross-reactivity. The anti-CD18 mAb for example were raised in mice against human CD18 and were found to cross-react with guinea pig cells. Since the target antigen in guinea pigs has not been identified by molecular methods, the specificity of the antibodies is not formally proven.

2.2. MAb directed against cell surface antigens without cluster of differentiation classification

Many attempts to generate mAb to surface antigens of guinea pig T cells were carried out in order to have a marker for the cell population under study or to analyze the consequences of antibody-binding for cellular functions. Not much attention was paid to the molecular nature of the recognized antigen or its orthologues in other species. Therefore many of these otherwise useful mAb cannot be classified following the cluster of differentiation nomenclature. These antibodies are listed in Table 2.

2.3. MAb interfering with cellular effector functions

Many of the mAb listed in Table 1 and Table 2 interfere with cellular effector functions by blocking binding to a physiological ligand or by mimicking interaction with this ligand. Therefore some of these antibodies are able to either block the physiological function of the target cell, whereas others show an activating effect. Antibodies directed against proteins encoded in the Major Histocompatibility Complex
2.4. MAb directed against antigens of the major histocompatibility complex

Several independent reports have described the production and utilization of anti-MHC antibodies (see Table 3 for references). Anti-class II mAb have been classified based on the expression and molecular characteristics of the recognized MHC protein, and on their capacity to interfere with class II restricted T cell responses [41] and [42]. The specificity of mAb B640 and MSgp 4 for MHC class I has been confirmed by cloning and expression of a guinea pig class I gene (unpublished). MAb potentially reactive with MHC antigens have been described based on immunohistological staining experiments [53], but the specificity has not been confirmed by additional methods.

2.5. Cell surface markers for leukocyte subpopulations

Although most of the mAb listed so far react with protein antigens on the surface of lymphocytes and macrophages, only some of them are appropriate to define populations and subpopulations within guinea leukocytes. Many membrane proteins are expressed on a variety of differentiation lineages and over long periods of cellular ontogeny. Strictly, only proteins which have a specific task at a specific state of cellular development should be expressed selectively on a group of cells with a defined function. In reverse, this means that each of these “markers” plays a role in the effector function of the cell or serves as receptor for growth factors or for signal transduction. The physiological ligands of these receptors might be a soluble mediator such as IL2 or a surface protein of an interacting cell such as MHC class I for the CD8 differentiation antigen.

2.5.1. Pan–leukocyte markers

Due to expression on all nucleated cells, anti-MHC class I mAb may be used to discriminate leukocytes from erythrocytes and thrombocytes in the blood and in cell suspensions from immunological organs. These antibodies however cannot be used to identify leukocytes on samples which contain other nucleated cells such as tissue sections or tumor cells. For the purpose of selectively staining leukocytes mAb H201 (anti-Leukocyte Common Antigen, CD45) can be used [54], because it provides strong staining of all leukocyte populations and low background on non-leukocytic cells. Anti-CD45 mAb specifically recognizing individual isofoms of the leukocyte common antigen have been described, which might turn out helpful for the discrimination between native and memory T cells subsets (Table 1).

2.5.2. Lymphocyte markers

In analogy to the rat system and in contrast to mice, Thy-1 (CD90) is expressed on T-and B-cells in guinea pigs, although staining of T-lymphocytes using mAb H154 is somewhat stronger than that of B cells, and germinal center B cells are completely negative [52]. Therefore antibodies against Thy-1 are not T cell markers in this species. Moreover fibroblasts, Langerhans cells and activated macrophages stain positive for Thy-1, very weak staining is observed on guinea pig erythrocytes. As in most other species, guinea pig Thy-1 is strongly expressed in the brain. In whole blood samples and single cells suspensions of spleen and lymph nodes anti-Thy-1 mAb might be useful to discriminate strong staining lymphocytes (B- and T cells) from weak or non-stained macrophages and granulocytes. Both in tissue sections and in single cell suspensions anti-Thy-1 mAb are helpful to dissect fibroblasts from epithelial or endothelial cells [52]. Based on the expression of Thy-1 we consider the guinea pig cell line 104C1 [55] as a fibroblast line whereas JH4 clone 1, which has a similar morphology is rather of epithelial or endothelial origin (Thy-1 negative). The L2C B cell leukemia line is negative for Thy-1, thus resembling germinal center B cells of the lymph node (Table 4).
2.5.3. T cells

Only very few mAb can be used as pan T-cell markers in the guinea pig, when compared to human or mouse cells. As outlined above Thy-1 is not restricted to T cells in the guinea pig and the expression of CD2 which is sometimes used as a T cell marker on human cells has not been identified in guinea pigs. Preliminary data from our lab have shown that rabbit antisera raised against recombinant CD3ε can be used to selectively stain T cells, but so far we have not been able to obtain a monoclonal anti-CD3 from immunized mice. MAb PC3/188 was raised against a conserved peptide of the CD3ε chain [56], which is also present on guinea pig T cells, but due to the cytoplasmic location of the epitope, the antibody only works with intracellular staining after permeabilization of the cell membrane. Therefore the best marker for T-lymphocyte in our hands has been mAb H159 [57]. Although there is weak staining of B cells, both populations can be fully separated using H159 both on tissue sections and by flow cytometry, the recognized antigen however has not been elucidated, although molecular data on the protein have been obtained. Based on these data we proposed H159 to be specific for the T cell receptor, but subsequent analysis of TCR proteins and comparison to the antigen detected by H159 argue against this hypothesis. Activated T lymphocytes and, to a minor extend, resting T cells and thymocytes, express la antigens in the guinea pig [58].

2.5.4. T cell subpopulations

The CD4- and CD8-positive subpopulations can be identified with several independently obtained mAb. The CD4-antigen is recognized by mAb H155 [59] and CT7, although the latter has initially been described as a pan-T cell marker [60] and cross-blocking studies with H155 have not been carried out so far.

The antibodies B607, CT6, and MSgp6 [47] and [60] all react with the alfa-chain of guinea pig CD8, which has meanwhile been confirmed by cloning of guinea pig CD8-alfa and transfection into mouse cells (unpublished). CT6 and B607 are of the same isotype (mouse IgG1) and stain quite strongly, whereas MSgp6 [61] is a mouse IgM antibody and shows weaker staining.

Examples for the use of anti-CD4 and anti-CD8 antibodies have been shown exemplarily [47]. In double staining experiments individual subpopulations of CD4 and CD8 single positive or double positive cells can be distinguished. The same mAb can be used to differentiate responding cells after CFSE-labeling and proliferation. Most of these antibodies have been used successfully to identify the recognized T cell subsets in cryosections [59], [60] and [61].

2.5.5. B cells

Surface IgM bearing B-lymphocytes can be reliably discriminated from other leukocyte populations by mAb 31D2 and B621, both directed against guinea pig IgM. After mice and rats were immunized with lymph node cells, B cell specific mAb were identified by immunohistochemistry and flow cytometry. Further analysis of the recognized antigen showed that 31D2 reacts both with soluble IgM and with surface IgM on B lymphocytes. This antibody may be used to deplete B cells from lymph node cells in vitro [62]. The specificity of B621 was deduced by partial cross-blocking with 31D2 and by the fact that pre-incubation with whole blood (containing soluble IgM) abolishes reactivity with lymphocytes. Most B cells as well as the ENL2C leukemia line are also stained by anti-MHC class II mAb 22C4 and MSgp8 [61].

2.5.6. Macrophages

Macrophages are represented by a variety of different cells types in many different organs. Most of these differentiation lineages have their own morphology and may possess quite different sets of surface antigens. In general most macrophages will express MHC class I antigens (strong staining) and class II antigens (variable), the leukocyte common antigen (strong), Thy1 (variable depending on activation state) and the antigen recognized by mAb H160 (unpublished). These surface antigens are shared with many other cells and can therefore not be considered as macrophage markers. Several authors have reported the generation of mAb specific for guinea pig macrophages with a broader or narrower expression profile on different macrophage lineages. MAb 342 [63] and MR-1 [64] provide specific and sensitive staining of a broad range of macrophages. Due to the intracellular expression of
the recognized antigen, MR-1 is better suited for immunohistochemistry experiments, whereas mAb 342 can be used for flowcytometry as well.

2.6. Cross-reactive mAb

A number of reports have been published on cross-reactive mAb which have been submitted to cluster of differentiation workshops for human cells and were simultaneously tested for reactivity with other species [65], [66] and [67]. Although this is potentially a fast and straightforward way to obtain useful mAb directed against guinea pig differentiation antigens, it has to be kept in mind that a positive reaction of a certain antibody with a guinea pig cell population does by no means guarantee that the homologous antigen is actually detected. A detailed characterization of the recognized antigen is urgently recommended. In the latest report 15 mAb out of 367 raised against human CD antigens showed clear cross-reactivity. Five of those however showed staining patterns in guinea pigs which were inconsistent with those reported in humans, and for some other antibodies no appropriate guinea pig target cells were available for further testing. For eight CD specificities it was concluded that, based on the staining pattern, the respective orthologue in the guinea pig was detected, a detailed analysis of the recognize antigen however has not been published so far. In a more successful approach three out of three mAb directed against human la antigens were found to react with la epitopes of strain 2 guinea pig cells [45]. Similarly, by checking available human anti-CD1 mAb several clones were found to cross-react with guinea pig CD1 antigens [68]. Rational design of the immunizing peptide based on sequence homology of CD-antigens between different species yielded mAb reacting with the target antigen of several animal species including guinea pigs [56].

3. Cell-lines and T cell hybrids

In the guinea pig system only a few cell lines showing autonomous growth in tissue culture or as transplantable tumors are available. Tissue culture for a variety of primary cells usually can be carried out following the protocols developed in other species.

3.1. Guinea pig tumor cell lines

Unfortunately no lymphocytic or myeloid cell line that can be grown in culture has been obtained from guinea pigs so far, restricting the options to immunize and screen with a highly homologous cell population for the generation of mAb. The available cell lines were mostly induced by treatment with chemical carcinogens and show a rather limited expression of cell surface antigens that can be detected with the available mAb. The fibroblast line 104C1 expresses MHC class I and Thy-1 antigens. The B cell leukemia ENL2C is positive for surface IgM, and class I and class II MHC. The variant BZL2C is an la negative subclone of the parental L2C cells [69].

3.2. T cell lines and clones

Alloreactive and antigen-specific T cell lines can be obtained be repeated restimulation of primed T lymphocytes in the presence of guinea pig IL2 or IL15. Expansion and cloning of these cells is achieved by following standard protocols [70], [71], [72] and [47]. Cells grown by repeated restimulation maintain cellular effector functions in vitro. To obtain highly pure populations of T cells in vitro, stimulated T lymphocytes can be grown in medium containing IL2 or IL15 without adding macrophages for antigen presentation. Some of these cultures have been grown for several months in our laboratory and continue to express T cell markers such as CD3 and the coreceptors CD4 or CD8 (unpublished observation).

3.3. T cell hybrids

We have shown, that guinea pig T cells can be fused with mouse and rat thymoma cell lines to produce T cell hybrids stably expressing guinea pig cell surface antigens [73]. Although these hybrids might be helpful to further characterize the binding specificity of mAb, they appear useless for functional studies, because none of these hybrids responded to polyclonal stimulation with the release of interleukin 2 (unpublished).
4. Outlook

Although the use of guinea pigs as experimental models apparently declines, there is still an increasing demand for research reagents in this species. The reasons for the lack of such tools have been discussed above, and the drawbacks of the guinea pig system in general, such as high cost, long gestation period, few inbred strains and no transgenic animals, are not likely to be resolved in the near future, which will in turn prevent many researchers from establishing guinea pig models in their lab. Therefore the community of “guinea pig researchers” will remain rather small compared to research groups employing mouse models. As a consequence it is rather unlikely that commercial suppliers will broaden their range of products in this category, so most of new reagents will have to be developed in scientific labs. Raising monoclonal and polyclonal antibodies to guinea pig proteins has become much easier with the sequencing of the guinea pig genome. We have used the information from public genome libraries to confirm the specificity of mAb against class I molecules and for recombinant expression of guinea pig cytokines and differentiation antigens (manuscript in preparation). These recombinant proteins have been used successfully to generate monoclonal and polyclonal antibodies, which represents a considerable improvement over the use of crude antigens such as whole cells or cellular supernatants as immunizing agents. Since guinea pigs, mice and rats are quite closely related on the phylogenetic tree, it is sometimes not possible to generate antibodies to certain guinea pig proteins in mice or rats. This obstacle has been overcome in the mouse system by using hamsters as hosts for the induction of an immune response [74]. We have found, that rabbits respond much better to certain recombinant guinea pig antigens than mice or rats (manuscript in preparation), and that rabbit polyclonal antibodies raised against guinea pig T cell differentiation antigens expressed in Escherichia coli can be used to specifically detect the protein on the cell surface by flow cytometry. With the recently described parental cell line developed in transgenic rabbits [75] it should be possible to produce mAb with the same specificity. The technique however is currently limited to commercial suppliers, because the parental line has not been made available to research laboratories.

Conflicts of interest statement: The authors have no conflicts of interest to declare
References

[27] Nuerberger E. Using animal models to develop new treatments for tuberculosis. Semin Respir Crit Care Med 2008;29(October (5)):542–51.
[53] Sato H, Inaba T, Kamiya H. Production of murine monoclonal antibodies to guinea pig leukocytes and immunohistochemistry of guinea pig skin exposed to Schistosoma mansoni. Hybridoma 1997;16(December (6)):529–36.
### Tables

**Table 1.** Monoclonal antibodies against cluster of differentiation antigens of the guinea pig.

<table>
<thead>
<tr>
<th>Cluster of differentiation</th>
<th>Recognized guinea pig antigen</th>
<th>Antibody name</th>
<th>Species</th>
<th>Isotype</th>
<th>Remarks</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD1</td>
<td>CD1b3</td>
<td>MSgp9</td>
<td>Mouse</td>
<td>IgG1</td>
<td>Expressed on B cells and thymocytes, gene cloned</td>
<td>[68]</td>
</tr>
<tr>
<td>CD1</td>
<td>CD1</td>
<td>BCD1b3.1</td>
<td>Mouse</td>
<td>IgG1</td>
<td>Raised against human CD1</td>
<td>[76]</td>
</tr>
<tr>
<td>CD1</td>
<td>Most CD1 isoforms</td>
<td>CD1F2/5E3</td>
<td>Mouse</td>
<td>IgG1</td>
<td>Specificity verified on transfected cell lines</td>
<td>[68]</td>
</tr>
<tr>
<td>CD1</td>
<td>All CD1 isoforms</td>
<td>CD1F2/6B5</td>
<td>Mouse</td>
<td>IgG1</td>
<td>Specificity verified on transfected cell lines</td>
<td>[68]</td>
</tr>
<tr>
<td>CD1</td>
<td>CD1b2, CD1b3, CD1b4</td>
<td>CD1F2/1B12</td>
<td>Mouse</td>
<td>IgG2a</td>
<td>Specificity verified on transfected cell lines</td>
<td>[68]</td>
</tr>
<tr>
<td>CD1</td>
<td>CD1b1</td>
<td>CD1.4-1D12</td>
<td>Mouse</td>
<td>IgG</td>
<td>Specificity verified on transfected cell lines</td>
<td>[68]</td>
</tr>
<tr>
<td>CD3</td>
<td>CD3 epsilon-chain</td>
<td>PC3/188A</td>
<td>Mouse</td>
<td></td>
<td>Intracellular staining only</td>
<td>[56]</td>
</tr>
<tr>
<td>CD4</td>
<td>CD4 coreceptor</td>
<td>H155</td>
<td>Rat</td>
<td>IgG2a</td>
<td>Inhibits T cell-proliferation</td>
<td>[59]</td>
</tr>
<tr>
<td>CD4</td>
<td>CD4 coreceptor</td>
<td>CT7</td>
<td>Mouse</td>
<td>IgG1</td>
<td></td>
<td>[60]</td>
</tr>
<tr>
<td>CD5</td>
<td>CD5</td>
<td>8BE6</td>
<td></td>
<td></td>
<td></td>
<td>[77], [78] and [79]</td>
</tr>
<tr>
<td>CD8</td>
<td>CD8 alfa-chain</td>
<td>B607</td>
<td>Mouse</td>
<td>IgG1</td>
<td>Binds to CD8a chain, gene cloned, specificity verified on transfected cell lines</td>
<td>[47]</td>
</tr>
<tr>
<td>CD8</td>
<td>CD8 alfa-chain</td>
<td>CT6</td>
<td>Mouse</td>
<td>IgG1</td>
<td>Binds to CD8a chain, gene cloned, specificity verified on transfected cell lines</td>
<td>[60]</td>
</tr>
<tr>
<td>CD8</td>
<td>CD8 alfa-chain</td>
<td>MSgp6</td>
<td>Mouse</td>
<td>IgM</td>
<td>Binds to CD8a chain, gene cloned, specificity verified on transfected cell lines</td>
<td>[47] and [61]</td>
</tr>
<tr>
<td>CD18</td>
<td>Beta-chain of leukocyte adhesion receptors (integrins)</td>
<td>IB4</td>
<td>Mouse</td>
<td></td>
<td>Raised against human CD18, cross-reactive to guinea pig</td>
<td>[81]</td>
</tr>
<tr>
<td>CD18</td>
<td></td>
<td>R15.7</td>
<td>Mouse</td>
<td></td>
<td>Raised against human CD18, cross-reactive to guinea pig</td>
<td>[82]</td>
</tr>
<tr>
<td>CD45</td>
<td>Leukocyte common antigen (LCA) all isoforms</td>
<td>H201</td>
<td>Rat</td>
<td>IgG2a</td>
<td>Binds all isoforms of gpLCA</td>
<td>[54]</td>
</tr>
<tr>
<td>CD45</td>
<td>Leukocyte common antigen (LCA) all</td>
<td>IH-1</td>
<td>Mouse</td>
<td></td>
<td>Similar to H201</td>
<td>[83]</td>
</tr>
<tr>
<td>Cluster of differentiation</td>
<td>Recognized guinea pig antigen</td>
<td>Antibody name</td>
<td>Remarks</td>
<td>Ref.</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>-------------------------------</td>
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<td>---------</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>CD45</td>
<td>LCA isoform-specific</td>
<td>IgG1</td>
<td>Reacts with most leucocytes and thymocytes</td>
<td>[83]</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>IH-2</td>
<td>Mouse</td>
<td>IgG1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD45</td>
<td>LCA isoform-specific</td>
<td>IgG1</td>
<td>Reacts with most leucocytes, but only with a minor portion of thymocytes</td>
<td>[83]</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>IH-4</td>
<td>Mouse</td>
<td>IgG1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD55</td>
<td>Decay accelerating factor</td>
<td>MCA44</td>
<td>Blocks complement regulatory activity on guinea pig erythrocytes</td>
<td>[84] and [85]</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mouse IgG1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD68</td>
<td>CD68 antigen</td>
<td>PM-1K</td>
<td>Mouse</td>
<td></td>
<td>[86]</td>
<td></td>
</tr>
<tr>
<td>CD90</td>
<td>Thy-1 antigen</td>
<td>H154</td>
<td>Co-stimulatory for T cell-proliferation, gene cloned</td>
<td>[52]</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rat IgG2a</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD90</td>
<td>Thy-1 antigen</td>
<td>CT4</td>
<td>Similar to H154, but mouse mAb, gene cloned</td>
<td>[52], [60] and [87]</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mouse IgG3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD90</td>
<td>Thy-1 antigen</td>
<td>MSgp2</td>
<td>Similar to H154 and CT4 but weaker staining</td>
<td>[52] and [88]</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mouse</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD90</td>
<td>Thy-1 antigen</td>
<td>167</td>
<td>Similar to MSgp2</td>
<td>[52]</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mouse</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Monoclonal antibodies directed against cell surface antigens without cluster of differentiation classification.

<table>
<thead>
<tr>
<th>Antibody-name</th>
<th>Isotype</th>
<th>Specificity</th>
<th>Remarks</th>
<th>Origin/reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>H159</td>
<td>rat IgG2a</td>
<td>T cells</td>
<td>Weak staining of B cells</td>
<td>[57]</td>
</tr>
<tr>
<td>5C3</td>
<td>ms IgM</td>
<td>Activated T cells</td>
<td>Blocks IL2-driven proliferation</td>
<td>[44] and [49]</td>
</tr>
<tr>
<td>CT5</td>
<td>ms IgG1</td>
<td>T cells and leukemic B cells</td>
<td>Considered as pan T cell marker, but also stains L2C B cell leukemia</td>
<td>[60]</td>
</tr>
<tr>
<td>MSgp7</td>
<td>ms IgM</td>
<td>T cells</td>
<td>Reacts with T cells and thymocytes</td>
<td>[48]</td>
</tr>
<tr>
<td>MSgp12</td>
<td>ms IgG1</td>
<td>T cells</td>
<td>Reacts with a subpopulation of T cells and thymocytes potential anti-CD4</td>
<td>[48]</td>
</tr>
<tr>
<td>31D2</td>
<td>ms IgG1</td>
<td>B cells (surface IgM)</td>
<td>Binds soluble and surface IgM, does not stimulate B cell proliferation</td>
<td></td>
</tr>
<tr>
<td>B621</td>
<td>rat IgG1</td>
<td>B-cells (surface Immunoglobulin)</td>
<td>Similar to 31D2</td>
<td></td>
</tr>
<tr>
<td>188</td>
<td>ms IgG2b</td>
<td>T- and B-cells</td>
<td>Inhibits T cell proliferation, stains weakly, good complement mediated cytotoxicity</td>
<td>[50]</td>
</tr>
<tr>
<td>H160</td>
<td>rat IgG2a</td>
<td>Leukocytes</td>
<td>Strongly inhibits T cell proliferation</td>
<td></td>
</tr>
<tr>
<td>342</td>
<td>ms IgM</td>
<td>Macrophages</td>
<td>Broad range of macrophages</td>
<td>[63]</td>
</tr>
<tr>
<td>305</td>
<td>ms IgG2a</td>
<td>Macrophages</td>
<td>Stains most macrophages</td>
<td>[63]</td>
</tr>
<tr>
<td>249</td>
<td>ms IgG1</td>
<td>Macrophages and platelets</td>
<td></td>
<td>[63]</td>
</tr>
<tr>
<td>MR-1</td>
<td>ms IgG1</td>
<td>Macrophages and monocytes</td>
<td>Intracellular antigen</td>
<td>[64]</td>
</tr>
<tr>
<td>VIIA1</td>
<td>ms IgG1</td>
<td>Macrophages</td>
<td>binds Fc Receptor 2</td>
<td>[89]</td>
</tr>
<tr>
<td>VIA2</td>
<td>ms IgG1</td>
<td>Macrophages</td>
<td>Binds Fc Receptor 1 and 2</td>
<td>[89]</td>
</tr>
</tbody>
</table>
### Table 3. MHC-specific monoclonal antibodies.

<table>
<thead>
<tr>
<th>Antibody-name</th>
<th>Isotype</th>
<th>Specificity</th>
<th>Remarks</th>
<th>Origin/reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSgp4</td>
<td>ms IgG1</td>
<td>MHC class I</td>
<td>Stains all immune cells except cortical thymocytes, immunoprecipitated a 45 kD antigen and beta-2-microglobulin</td>
<td>[48] and [47]</td>
</tr>
<tr>
<td>B640</td>
<td>ms IgG1</td>
<td>MHC class I</td>
<td>Similar to MSgp4, specificity confirmed by binding to recombinant class I gene on transfected cells</td>
<td></td>
</tr>
<tr>
<td>HUSM-20</td>
<td>ms IgG2a</td>
<td>MHC class I</td>
<td>Specificity appraised from immunohistological staining</td>
<td>[53]</td>
</tr>
<tr>
<td>HUSM-41</td>
<td>ms IgG2a</td>
<td>MHC class I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSgp8</td>
<td>Ms IgG3</td>
<td>MHC class II</td>
<td>Good for tissue staining</td>
<td>[48]</td>
</tr>
<tr>
<td>25E3</td>
<td>ms IgG1</td>
<td>MHC class II</td>
<td>Inhibits T cell-proliferation</td>
<td>[41], [42] and [58]</td>
</tr>
<tr>
<td>22C4</td>
<td>ms IgG1</td>
<td>MHC class II</td>
<td>Inhibits T cell-proliferation</td>
<td>[41], [42] and [58]</td>
</tr>
<tr>
<td>CI-13.1</td>
<td>ms IgG1</td>
<td>MHC class II</td>
<td>Specific for strain 13 and outbred gpips, negative on strain 2 cells</td>
<td>[60]</td>
</tr>
<tr>
<td>HUSM-19</td>
<td>ms IgG2a</td>
<td>MHC class II</td>
<td>Specificity appraised from immunohistological staining</td>
<td>[53]</td>
</tr>
<tr>
<td>HUSM-45</td>
<td>ms IgG2a</td>
<td>MHC class II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HUSM-49</td>
<td>ms IgG2a</td>
<td>MHC class II</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 4. Guinea pig tumor cell lines.

<table>
<thead>
<tr>
<th>Designation</th>
<th>Morphology</th>
<th>Source/organ</th>
<th>Growth</th>
<th>ATCC-number</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>104 C1</td>
<td>Fibroblast</td>
<td>LUNG</td>
<td>Adherent</td>
<td>CRL-1405</td>
<td>[55]</td>
</tr>
<tr>
<td>JH4 clone 1</td>
<td>Fibroblast/epithelial</td>
<td>FETUS</td>
<td>Adherent</td>
<td>CCL-158</td>
<td>[90]</td>
</tr>
<tr>
<td>GPC 16</td>
<td>Epithelial</td>
<td>Colon</td>
<td>Adherent</td>
<td>CCL-242</td>
<td>[91]</td>
</tr>
<tr>
<td>EN L₂C</td>
<td>Lymphoid</td>
<td>Blood</td>
<td>In vivo/leukemia</td>
<td>n.a.</td>
<td>[92] and [93]</td>
</tr>
<tr>
<td>BZ L₂C</td>
<td>Lymphoid</td>
<td>Blood</td>
<td>In vivo/leukemia</td>
<td>n.a.</td>
<td>[94]</td>
</tr>
<tr>
<td>Line-10</td>
<td>Variable</td>
<td>Liver</td>
<td>In vivo/ascites</td>
<td>n.a.</td>
<td>[95]</td>
</tr>
</tbody>
</table>