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The Porcine 2A10 Antigen Is Homologous to Human CD163 and Related to Macrophage Differentiation

Carmen Sánchez, Nieves Domínech,* Jesús Vázquez, † Fernando Alonso,* Angel Ezquerra,* and Javier Domínguez 3 *

The mAb 2A10 recognizes a 120-kDa protein with sequence homology to the human CD163 and whose expression is restricted to the cells of the porcine monocyte/macrophage lineage. While most of tissue macrophages express high levels of 2A10 Ag, bone marrow cells and a subset of blood monocytes are negative for this marker. The percentage of 2A10+ blood monocytes ranges between 5–50% depending on the donor. The phenotypic analysis indicates that these cells are more similar to mature macrophages than 2A10− monocytes. 2A10+ monocytes express higher levels of swine histocompatibiltiy leukocyte Ag II, CD16, and the adhesion molecules very late Ag-4 (CD49d) and LFA-1 (CD11a) than 2A10− monocytes, while CD14 and SWC1 expression is lower. Both monocyte subsets also differ in their functional capabilities. 2A10+ monocytes induce a greater alloengenic response on T lymphocytes than 2A10− cells. LPS-stimulated 2A10+ and 2A10− monocytes both produce proinflammatory cytokines (TNF-α and IL-1β), but antiinflammatory IL-10 is only detected on the latter population. When 2A10− monocytes were cultured in medium containing pig serum, they acquired some phenotypic features of 2A10+ cells, expressing the 2A10 Ag. In contrast, when they were cultured in the presence of L929 supernatant as a source of GM-CSF, the 2A10 Ag expression remained low, scarcely increasing over basal levels. 2A10+ cells cultured with pig serum developed features that resemble monocyte-derived dendritic cells. These results indicate that 2A10+ monocytes could constitute a cell population in a more advanced maturation stage than 2A10− circulating monocytes. The Journal of Immunology, 1999, 162: 5230–5237.

The mononuclear phagocyte system is a very heterogeneous cell population dispersed all over the organism and is involved in a number of homeostatic, inflammatory, and immunological events (1). Their wide distribution provides an immediate defense against foreign elements. Macrophages exhibit an extensive range of phenotypes that might account for their remarkable functional plasticity (2). The origin of such heterogeneity is still partially unknown, and several models have been proposed (2, 3).

Macrophages originate from precursor cells in the bone marrow which sequentially develops into monoblasts, promonocytes, and monocytes. Monocytes enter the blood circulation from which they migrate to the various tissues, where they undergo further differentiation transforming into exudate macrophages. The maturation process ends when these exudate macrophages turn into resident macrophages. There is also evidence that some tissue resident macrophages are renewed by local proliferation. In vitro differentiation of peripheral blood monocytes in the presence of serum has been extensively used as a model system to investigate macrophage development (4). These studies have evidenced the high plasticity of the monocyte/macrophage lineage, since other cell types, in addition to macrophages, may be obtained from monocytes depending on the culture conditions (i.e., dendritic cells (DC)4 in the presence of GM-CSF and IL-4) (5).

The expression of surface markers has been widely used to identify different maturation stages within a particular lineage and to evaluate lineage-relationships among different cell types. In the human system, two major subsets of monocytes with distinct functional properties have been defined based on the expression of CD14, CD16, and CD64 Ags. Compared with CD64+CD14+CD16− cells, CD64low/CD14+CD16+ cells express higher levels of MHC class II Ags and adhesion molecules (6, 7). CD16− monocytes were also reported to have a lower phagocytic activity and capacity of production of oxygen radicals than CD16− monocytes (7, 8). Both subsets also differ in their accessory cell capacity and in their pattern of cytokine expression following LPS stimulation (7, 9, 10). All these findings have led to consider CD16+ monocytes in a more mature stage than CD16− cells (11).

The study of the swine immune system has been subject of considerable interest because of its importance in the design of better vaccines for controlling infectious diseases in this economically important livestock species. This interest has been spurred in the last years by the advances on xenograft transplantation and the potential use of this species as a source of organs. However, studies on the swine monocyte/macrophage lineage have been limited by the lack of specific markers. Although a significant number of mAbs have been raised against porcine mononuclear phagocytes,

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4 Abbreviations used in this paper: DC, dendritic cells; PAM, porcine alveolar macrophage; IF, immunofluorescence; SLA, swine histocompatibility leukocyte Ags; SRCR, scavenger receptor cysteine-rich; MACS, magnetic cell separation system; RT, reverse transcriptase; VLA-4, very late Ag-4.
most of them are not lineage specific or recognize only a small subpopulation of macrophages. However, we have produced recently a panel of mAbs that recognize molecules whose expression is restricted to swine macrophages. One of these mAbs, named 2A10, seems to be of potential utility for studying the process of macrophage differentiation in swine. This mAb reacts with a 120-kDa monomeric glycoprotein, which is strongly expressed by the majority of tissue macrophages and at lower levels by a subset of monocytes (12).

In the present study, we have examined the heterogeneity of blood monocytes based on 2A10 expression with respect to the coexpression of surface molecules that are critical for effective monocyte function and in terms of their capacity as accessory cell to induce MLR and synthesize cytokines. Amino acid sequence analysis of peptides derived from the 2A10 molecule shows a high similarity to the indications of the manufacturer. PAM lysates were obtained from 3 × 10^6 cells. Lysis was performed in 1% Nonidet P-40, 50 mM Tris, pH 8, 150 mM NaCl, 5 mM EDTA, 0.1 mM PMSF, 10 μg/ml aprotinin, and 10 mM iodoacetamide (lysis buffer) at 10^6 cells/ml for 1 h at 4°C. Lysates were precleared by incubation for 24 h with normal mouse IgG-coupled Sepharose beads. Then, precleared lysates were incubated for additional 24 h with 2A10 mAb-coupled beads. After that, beads were washed several times with lysis buffer and, finally, with PBS. The adsorbed fraction was eluted from the beads by adding 50 mM ethyleneamine, pH 11. The eluted fraction was dialyzed against 10 mM ammonium bicarbonate, pH 8, and concentrated by lyophilization. Then, it was subjected to SDS-PAGE in 12% polyacrylamide and electrotransferred onto a polyvinylidene difluoride membrane (Immobilon-P, Millipore, MA, USA). The main band, with an apparent molecular mass of 120 kDa, was excised and subjected to automated Edman degradation to obtain the N-terminal sequence. Internal peptides were obtained from protein digestion with trypsin, separated by HPLC, and also subjected to Edman degradation on an Applied Biosystem 473-A pulse liquid phase protein sequencer (Applied Biosystem, Foster City, CA). Peptide identities were searched on SwissProt, EMBL, and GeneBank databases using the GCG analysis program (17).

**Materials and Methods**

**Cells**

Large-White outbred pigs were used as cell donors. Porcine alveolar macrophages (PAM) were collected by alveolar lavage as described by González et al. (15). Bone marrow cells were obtained after perfusing ribs with PBS. Red cells and nonviable cells were removed by centrifugation at 400 × g for 10 min on lymphocyte separation medium at 1.077 g/ml (BioWhittaker, Walkersville, MD). For long-term cultures, monocytes were placed on Teflon jars at 2 × 10^5 cells/ml in RPMI 1640 medium containing 10% FCS, 2 mM L-glutamine, 5 × 10^{-5} M 2-ME, 20 mM HEPES, 30 μg/ml gentamicin (complete culture medium), and either 20% pig serum or 20% L929 cell culture supernatant (as a source of GM-CSF) (16).

**Abs**

Murine anti-swine leukocyte Ag mAbs 2A10/11 (IgG1), BLH18 (IgG3), CD141a, 2F4/11 (IgG1, CD11b), and 1F12 (IgG3, swine histocompatibility leukocyte Ag (SLA) II DR) were produced in our laboratory. Human CD14 mAb TU¨ K-4 (IgG 2a ) was obtained from Dako (Glostrup, Denmark). Human anti-swine leukocyte Ag (SLA) II DR were produced in our laboratory. Human CD11a, CD16, CD11b, and 2A10 mAb were a gift from Y. B. Kim (Pittsburgh University, Pittsburgh, PA). SWC1 mAb 76-6-7 (IgM) and SWC3 mAb G7 (IgG1) and SWC9 mAb PM18/7 (IgG1) were a gift from Y. B. Madrid (Universidad Autónoma Madrid, Madrid, Spain). Swine CD16 bromide-activated Sepharose 4B beads (Pharmacia, Uppsala, Sweden), acrylamide, and 2 A¹ 10 mAb were coupled to 1 ml of cyanogen bromide-activated Sepharose 4B beads (Pharmacia, Uppsala, Sweden).

**Immunofluorescence assays**

Immunofluorescence (IF) analyses were performed with 10^6 cells (per Ab) on PBMC and bone marrow cells or with 10^5 cells when monocytes and PAM were used. For single-color IF, cells were incubated with 50 μl of hybridoma supernatant for 30 min at 4°C. Then, cells were washed with PBS containing 0.1% BSA and 0.01% NaN3 (fluorescence buffer) and incubated with 5 ml of 2A10 hybridoma supernatant for 30 min at 4°C. Then, cells were washed again and incubated for 20 min at 4°C with PE-conjugated goat Fr(ab)2 anti-mouse Ig (Dako) for 30 min at 4°C. Cells were then washed in fluorescence buffer, fixed in 0.1% paraformaldehyde, and analyzed in a FACScan flow cytometer (Becton Dickinson, San Jose, CA). For two-color IF, after labeling with the first mAb as described above, cells were incubated with a biotin-labeled second mAb for 30 min at 4°C. Then, cells were washed again and incubated for 20 min at 4°C with PE-conjugated streptavidin (Southern Biotechnology Associates, Birmingham, AL). After washing, cells were fixed and analyzed as described above. Before analysis, unstained control cells and FITC- and PE-stained cells were run to set proper compensation and to define quadrants.

**Sorting of 2A10^+ and 2A10^- monocytes**

Blood monocytes were magnetically isolated by using the VarioMACS cell sorting technique (Miltenyi Biotec, Bergisch-Gladbach, Germany). Briefly, 5 × 10^6 PBMC were incubated with 5 ml of 2A10 hybridoma supernatant for 45 min on ice. Then, cells were washed with PBS containing 5% FCS and 2 mM EDTA (magnetic cell separation system (MACS) buffer) and incubated with 1 ml of goat anti-mouse IgG magnetic microbeads for 15 min on ice. After washing with MACS buffer, PBMC were passed through a MACS separation column (VS^+ positive selection column) and magnetically labeled cells (2A10^+ fraction) were collected. The effluent negative fraction was then incubated with 5 ml of 74-22-15 (anti-SWC3) hybridoma supernatant and MACS microbeads as described above. PBMC were passed through the separation column, and 2A10^+ SWC3^- cells were harvested (2A10^- fraction). Isolated cells were left in complete medium for 12 h before used.

**Table I. Characteristics of mAbs used in the study**

<table>
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<tr>
<th>mAb</th>
<th>Specificity</th>
<th>Molecular Mass (kDa)</th>
<th>PAM</th>
<th>MON</th>
<th>GRAN</th>
<th>LYMP</th>
<th>PLAT</th>
<th>BMC</th>
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<td>CD163</td>
<td>120</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+/–</td>
</tr>
<tr>
<td>BLH18</td>
<td>CD11a</td>
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<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>HP2/1</td>
<td>CD49d</td>
<td>150</td>
<td>+</td>
<td>+</td>
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<td>ND</td>
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<td>ND</td>
</tr>
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<td>40</td>
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<tr>
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<td>SWC9</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>

MON, blood monocytes; GRAN, granulocytes; LYMP, lymphocytes; PLAT, platelets; BMC, SWC3^+ bone marrow cells.

+++, >90% of positive cells; +++, 50–90%; +, 10–50%; +/–, 5–10%; –, <5%.
MLR assays
A constant number of $3 \times 10^6$ allogeneic PBMC were incubated with graded numbers of magnetically isolated 2A10$^+$ or 2A10$^-$ monocytes. Monocytes were irradiated (3000 rad in a $^{137}$Cs source) and then cocultured with allogeneic PBMC for 5 days in 10% pig serum supplemented medium. Experiments were performed in 96-well plates in triplicate. T cell proliferation was measured by $[^{3}H]$Tdr (Amersham, Little Chalfont, U.K.) incorporation on day 5 of culture during the last 16 h. After freezing and thawing of the microplate cultures, the cells were harvested onto filters by using a multiharvester system (Tomtec, Orange, CT), and $[^{3}H]$thymidine uptake was subsequently measured in a microplate scintillation counter (Wallac, Turku, Finland).

Cytokine detection
A total of $2 \times 10^6$ 2A10$^+$ and 2A10$^-$ isolated monocytes were incubated for 6 h on 6-well plates with or without LPS at 1 $\mu$g/ml. Then, total RNA was extracted by using 100 $\mu$l of Tripure isolation reagent (Boehringer Mannheim, Indianapolis, IN) based on the method described by Chomczynski and Sacchi (18). First strand cDNA was prepared from 5 $\mu$g of total RNA, previously denatured by heating 2 min at 65°C and immediately placed on ice, with 5 $\mu$l of reverse transcriptase (RT) reaction mix containing 10 $\mu$l Moloney murine leukemia virus RT buffer (Epitector, Madison, WI), 10 mM DTT, 0.5 mM oligo(dT), 50 $\mu$M each of dNTPs, 12.5 U Moloney murine leukemia virus RT (Epitector), and 20 U RNasin (Promega, Madison, WI). The reaction mixtures were made up to 50 $\mu$l with RNase free water and incubated for 1 h at 37°C. For PCR, a variable amount of the cDNA (typically 2.5 $\mu$l) was used in a total volume of 25 $\mu$l with 5 $\mu$l MgCl$_2$ (as empirically determined for the different oligonucleotide pairs), 50 $\mu$M each of dNTPs, 5 $\mu$l with 50 units of reverse transcriptase (RT) reaction mix containing 10 $\mu$l Tripure isolation reagent (Epitector), and 20 $\mu$l RNasin (Promega, Madison, WI). The mixture was amplified on a GeneAmp PCR System 9600 (Perkin-Elmer, Norwalk, CT). The PCR products were electrophoresed on 2% agarose gels containing ethidium bromide and transferred onto a positively charged nylon membrane (Hybond-N, Amersham, Little Chalfont, U.K.), which was then exposed to X-ray film. Sequence comparison of 2A10 Ag with N-terminus from other scavenger receptor cysteine-rich (SRCR) domain-containing proteins showed lower homologies: i.e., 40% to human CD5 (20), CD6 (21), and bovine WC1.1 Ag (22), 30% to bovine CD5 (23), and 20% to the sea urchin speract receptor (24).

Three internal sequences from tryptic peptides of 2A10 Ag were also obtained: S38 (Leu-Glu-Val-Phe-Ser-Gly-Gly-Glu), S7 (Gly-Ser-Glu-Val-Met/Cys-Arg), and S8 (Gly-Asp-Ile-X-Pro-Ala-Ser, Table III). An identity of 62.5% was observed between peptide S38 and two distinct sequences from the third and eighth SRCR domain of M130 Ag. Peptide S7, considering the cysteine on the fifth position, shared 66.6% of identity with a peptide from the M130 seventh SRCR domain. Finally, peptide S8 showed a degree of identity of 87.5% with an internal sequence on the M130 eighth SRCR domain. No other sequences included on the SwissProt or GeneBank databases showed a significant homology to the swine 2A10 Ag peptides.

2A10 Ag expression on porcine myeloid cells
The distribution of 2A10 Ag has been previously shown to be restricted to the swine monocyte/macrophage lineage, with the highest levels of expression found in tissue macrophages (12). Here, we compared the 2A10 expression on cells that can be considered as three different maturation stages along the macrophage differentiation pathway: bone marrow myeloid precursors, monocytes, and PAM. No other markers for hemopoietic precursors in the bone, bone marrow myeloid precursors were selected by the expression of SWC3 Ag. This molecule is highly expressed along all stages of the myeloid differentiation pathway (25). Monocytes were selected from PBMC on the basis of their SWC3 expression and by their light scatter.
characteristics. PAM were obtained from broncoalveolar lavage, and 95% of resultant cells bore the SWC3 marker. As shown in Fig. 1, the expression of 2A10 Ag increased throughout the maturation of the macrophage lineage. Thus, while 2A10 molecules were practically undetectable on freshly isolated bone marrow cells, almost 100% of PAM were positive for this marker. In monocytes, two subpopulations could be distinguished, one negative and the other expressing medium levels of 2A10 Ag. The percentage of 2A10 monocytes varied among animals, ranging between 5–50% (data not shown).

Phenotype of 2A10 and 2A10 blood monocytes

The above data prompted us to further characterize the phenotype exhibited by monocytes with regard to the 2A10 Ag expression. Thus, freshly isolated PBMC were double-stained with mAb 2A10 vs mAbs against CD14, CD16, SLA II, SWC3, SWC9, CD11a, CD11b, and very late Ag-4 (VLA-4) markers (Fig. 2). Monocytes were gated from PBMC by their light scatter properties. Results showed several phenotypic differences among 2A10+ and 2A10− monocytes. 2A10+ cells expressed higher levels of MHC class II molecules (SLA II) than 2A10− monocytes. Expression of CD16, CD11a, and VLA-4 markers was also higher on 2A10+ cells, although the differences with 2A10− cells were not so pronounced as in the case of SLA class II. It is worthy to note that the whole population of porcine monocytes bears the CD16 marker, in contrast to human monocytes. On the other hand, the 2A10+ population showed lower levels of the CD14 receptor than 2A10− monocytes. No major differences were found between both cell subsets on SWC3 and CD11b expression. None of the monocytes subpopulations were positive for SWC9, CD1, CD3, CD4, CD8, IgM, and CD45RA markers (data not shown).

Isolation of 2A10+ and 2A10− blood monocytes

2A10+ and 2A10− monocytes were magnetically isolated to evaluate their morphological and functional characteristics. 2A10+ monocytes were first isolated from PBMC by mAb 2A10 binding. Then, 2A10+ monocytes were selected from the remaining cells by their reactivity with anti-SWC3 mAb 74-22-15. When analyzed by light microscopy, both cell subpopulations showed typical features of monocytes (Fig. 3A), with heterogeneous nuclei of lobulated, reniform, or round shape and dense clumps of chromatin. The 2A10+ cells were slight but significantly larger, with an average size of 16.6 ± 1.6 μm over 14.8 ± 1.8 μm of 2A10− monocytes (p < 0.01). Fig. 3B shows the forward light scatter characteristics of 2A10+ and 2A10− cells 12 h after isolation and a perceptible difference in their scatter parameters (higher for 2A10+ cells) could be observed.

Differentiation of cultured 2A10+ and 2A10− monocytes

Because the phenotype of 2A10+ monocytes appeared to be reminiscent of that of mature tissue macrophages, we tested next whether isolated 2A10− monocytes could differentiate and express a similar surface phenotype after culture with either 20% pig serum or 20% L929 cell-conditioned medium. Both media have been shown to support the maturation of cultured swine monocytes (26, 27). The results were compared with those obtained from 2A10+
monocytes under the same culture conditions. Fig. 4 shows the number of viable cells of these subsets along a 5-day culture with homologous serum or L929 supernatant. We found no major variation of 2A10\(^{+}\) cell numbers on both conditions. In contrast, we observed larger differences on cultured 2A10\(^{-}\) monocytes, with a pronounced cell death in the cultures with pig serum, and a detectable increase of viable cells in those with L929 supernatant.

The phenotype of 2A10\(^{+}\) and 2A10\(^{-}\) monocytes was examined throughout these cultures by single-color IF, starting 12 h after monocyte isolation (Fig. 5). On 2A10\(^{-}\) monocytes, a decrease of CD14 and SWC1 surface levels was observed after 5 days of culture with pig serum, together with a slight increase of CD16 and SLA II Ags and a higher induction of 2A10 and SWC9 expression (up to 10 times over 12 h culture levels). In contrast, on 2A10\(^{+}\) cells maintained with L929 supernatant, only a small increase of 2A10, and none of SLA II and SWC9 was observed along the time of culture, although a similar reduction of CD14 and SWC1 levels did occur. Likewise, culture of 2A10\(^{-}\) monocytes with pig serum led to an increased expression of SLA II, 2A10, and SWC9 and a reduction of CD14 and SWC1 expression. Finally, 2A10\(^{-}\) monocytes cultured with L929 supernatant showed a reduction of CD14, SWC1, and SLA II levels, with only a small induction of SWC9 expression on these cultures.

Cultured 2A10\(^{-}\) monocytes developed features characteristic of macrophages on the two employed conditions, with eccentric rounded nuclei, larger cell size, and smaller ratio nucleus/cytoplasm than freshly isolated monocytes (Fig. 6). 2A10\(^{+}\) monocytes became also morphologically similar to macrophages when cultured with L929 supernatant, but after culture with homologous serum, they developed an stellate morphology with cytoplasmic projections (Fig. 6). This last cell population was not homogeneous; a small percentage of cells showed typical macrophage morphology (not shown).

Stimulatory capacity of 2A10\(^{+}\) and 2A10\(^{-}\) monocytes in allogeneic MLR

Because of the different phenotype exhibited by 2A10\(^{+}\) and 2A10\(^{-}\) monocytes, particularly with regard to the SLA II molecules expression, we tested the ability of both subpopulations to act as accessory cells in an allogeneic MLR.
constant number of allogeneic PBMC, and the lymphoproliferative response was measured at day 5 of culture (Fig. 7). At lower cell concentrations (6,000–12,000 cells/well), 2A10+ monocytes showed to be up to 3–4 times more potent accessory cells than 2A10− monocytes, whereas at the highest cell concentration tested (25,000 cells/well), both populations induced a similar response.

**Cytokine expression in 2A10− and 2A10+ monocytes**

Magnetically isolated 2A10+ and 2A10− monocytes (>95% purity) were cultured for 6 h with or without LPS at 1 μg/ml. Then, total RNA was extracted and a reverse transcription followed by PCR was performed using specific primers for TNF-α, IL-1α, IL-10, and the housekeeping gene GAPDH. Fig. 8 shows the results of a representative experiment. mRNA for the proinflammatory cytokines tested, TNF-α and IL-1α, was clearly induced upon LPS stimulation in both 2A10+ and 2A10− cells. Basal levels of these cytokines varied among experiments. In contrast, mRNA induction for the antiinflammatory cytokine IL-10 was only detected after LPS stimulation of 2A10− monocytes.

**Discussion**

Heterogeneity within the monocyte/macrophage system has been defined on the basis on their phenotypic, morphologic, or functional characteristics. Early studies have identified subpopulations of blood monocytes based on differences in sedimentation or adherence properties (28, 29), but more conclusive results have been obtained by cell phenotyping (7, 8). Nevertheless, none of the markers employed to define monocyte subsets (Fcγ receptors CD16, CD64, CD14, CD43) (7, 8, 30) is strictly specific of the monocyte/macrophage lineage. In this study, we define two subpopulations of swine blood monocytes based on the expression of a recently reported marker, the Ag recognized by mAb 2A10 (12), whose distribution is restricted to the monocyte/macrophage lineage.

2A10 Ag can be considered as the porcine orthologue of human M130 Ag (19), recently assigned to CD163 at the 6th Human Leukocyte Typing Workshop, as peptide sequence identity between both Ags (62–87%) is within the range of interspecies molecular homology. Structurally, human M130 belongs to the scavenger receptor superfamily (19), and it contains nine SRCR domains. The SRCR domain has about 110 amino acid residues with six to eight conserved cysteines involved in intradomain disulfide bonds (31). Members of this ancient superfamily of proteins have been found from the lowest phylum of Metazoa (32) to mammals (19–23), and most of them seem to be involved in host defense (31). Despite of the conservation of SRCR domains along the evolution, human M130 Ag and no other reported proteins of the same superfamily showed significant similarity to peptides of the porcine 2A10 molecule.

Additional data support the identity between M130 and 2A10 Ags. Both markers seem to be specific of the monocyte/macrophage lineage, and no expression on other myeloid cells has been observed. Tissue distribution of both molecules is very similar; they are strongly expressed by macrophages of the red pulp of the spleen, the interfollicular and sinus macrophages of lymph nodes, the cortical macrophages of the thymus, and the Kupffer cells of the liver, while no reactivity is found on macrophages in follicles and the marginal zone of the spleen (12, 33–35). In our study, porcine 2A10 Ag appears to be a differentiation marker of the macrophage lineage: it is not detectable on freshly isolated bone marrow cells, being expressed at intermediate levels on blood monocytes and at high levels on alveolar macrophages. Likewise, M130 expression increases along the macrophage differentiation pathway, being weak on blood monocytes and strong on tissue macrophages. Furthermore, monoblastic (U937) and myelomonocytic (HL60) cell lines are negative for this marker (33, 34). On the other hand, the apparent molecular mass reported for M130 Ag varies from 130 kDa to 140 kDa, depending on the authors (33, 35); whereas 2A10 Ag shifts from 120 kDa before reduction to 150 kDa after reduction, this feature is indicative of the presence of intrachain disulfide bonds (12) and agrees with the characteristics of SRCR domains. Finally, although these molecules are expressed on the cell surface, substantial amounts of both of them are located intracellularly. Cloning of the 2A10 Ag is currently in progress, which will aid to define its homology with M130 molecule.

The two subpopulations of swine monocytes defined on the basis of the expression of 2A10 Ag phenotypically resemble the human monocyte subsets CD14+CD16− (2A10− monocytes) and CD14+CD16+ (2A10+ monocytes) (6, 8, 11). In swine, the whole population of blood monocytes bears the marker CD16, but 2A10+ monocytes express it at higher levels than 2A10− cells. Moreover, CD14 expression is clearly diminished on 2A10+ in relation to 2A10− monocytes. Additional markers such as SLA II, VLA-4, and LFA-1 are increased on 2A10+ monocytes, like on human CD16+ monocytes. No significant differences on CD11b levels were observed between 2A10− and 2A10+ monocytes, although its expression is decreased on human CD16+ monocytes (6, 36). In
fact, unlike human CD11b, whose expression is confined to well-differentiated myelomonocytic cells (37, 38), swine CD11b is expressed on almost 100% of SWC3+ bone marrow cells, and its level is maintained along the stages of differentiation of the swine monocyte/macrophage lineage (data not shown).

Both porcine 2A10+ and human CD16+ monocyte subsets represent a minor percentage within the circulating monocyte population (8). Interestingly, both subsets fail to produce IL-10 in response to LPS stimulation (10). IL-10 is one of the best known inhibitory cytokines of the monocyte/macrophage activation (39). Its role as an antiinflammatory factor is well documented, as it inhibits the synthesis of IL-1, IL-6, TNF-α, and GM-CSF on LPS-stimulated monocytes (40). Furthermore, it has an inhibitory effect on IFN-γ production by Th1 cells, partly through the inhibition of IL-12 synthesis by accessory cells, which contributes to the suppression of cellular responses (41). The absence of IL-10 production by human CD16+ monocytes has been interpreted as though these cells are of a proinflammatory type (10). Their high levels of LFA-1 and VLA-4 integrins (6) support this hypothesis, because it might allow a rapid monocyte transvasation from the blood to the inflamed tissues. Both characteristics are also present on 2A10+ monocytes, which have been also shown to produce inflammatory cytokines (TNF-α and IL-1) after bacterial LPS stimulation.

However, there are some significant differences between swine 2A10+ and human CD16+ monocytes. Human CD16+ monocytes are smaller and denser cells than CD16+ monocytes, whereas 2A10+ are slightly larger than 2A10+ monocytes. Further differences come from functional studies on CD16+ monocytes. Thomas and Lipsky (9) reported a low accessory and APC capacity for this human monocyte subset. In contrast, our results show that 2A10+ monocytes are strong stimulatory cells in MLR, which is in accordance with the data reported by Graje-Griebenerow et al. on human CD64+ monocytes (7). These cells share phenotypical characteristics with swine 2A10+ and human CD16+ monocytes. Isolation procedures might be the cause of such differences, as the three populations have been separated by sorting with distinct mAbs. However, based on those morphological and functional discrepancies, we cannot discard that 2A10+ monocytes represent a subpopulation different from human CD16+ monocytes.

Compared with 2A10+ monocytes, the phenotype of 2A10+ monocytes is closer related to that of mature macrophages. Thus, PAM express high levels of 2A10, CD16, and SLA II molecules, whereas their CD14 levels are lower than those of 2A10− monocytes (data not shown). On the other hand, as has been mentioned before, 2A10+ monocytes express higher levels of the integrins LFA-1 and VLA-4, appearing to be better endowed to leave the blood and reach the tissues. Therefore, they might represent the precursors of monocyte-derived tissue macrophage populations.

When magnetically isolated 2A10− monocytes were cultured in vitro with porcine serum, they developed a phenotype close to that of 2A10+ monocytes, reinforcing the idea that both subsets represent two different stages of maturation. On those cultured cells, the expression levels of 2A10, CD16, and SLA II Ags increased, but CD14 expression diminished. However, the levels of tested integrins (LFA-1, CD11b, and VLA-4) did not show significant changes during the culture (not shown). Two markers of swine macrophage differentiation, SWC9 and SWC1, were also analyzed. Within the porcine monocyte/macrophage lineage, SWC9 is expressed exclusively on mature macrophages, while SWC1 expression is remarkably higher on monocytes than on tissue macrophages (42). As expected, serum-induced monocyte maturation was accompanied by an increment of SWC9 and a reduction of SWC1 expression, both in 2A10− and 2A10+ cells. 2A10− monocytes cultured with L929 supernatant showed an intermediate phenotype, particularly in relation to 2A10 and SWC9 Ags, whose up-regulation seems to be mostly dependent on factors present in the serum.

One striking feature of 2A10+ monocytes is the morphology that they acquire after a few hours of culture with pig serum. On these conditions, while 2A10− monocytes are driven to a macrophage morphology, 2A10+ cells develop features that closely resemble monocyte-derived DC (43). It is worthy to note that these cells were grown on suspension cultures, therefore the cytoplasmic projections that they develop cannot be attributed to adherence processes in a solid substrate. Functional DCs can be generated from purified human blood monocytes after GM-CSF plus IL-4 treatment, and their morphologic changes (43) are similar to those observed in serum-cultured 2A10+ monocytes. Unfortunately, most of the markers employed to characterize DCs (i.e., CD40, CD83, CD86) are unavailable in the porcine system. However, the up-regulation of MHC class II and the loss of CD14 expression that we find on serum-cultured 2A10+ monocytes are characteristics of human monocyte-derived DCs (43, 44). Furthermore, preliminary results reveal that CD1, a MHC class I-like molecule whose expression is induced on PBMC-derived DCs (43, 45), is expressed on the membrane of 2A10+, but not of 2A10− monocytes (not shown). As mentioned above, isolated 2A10+ monocytes possess a great stimulatory capacity in MLR, which could be correlated, at least in part, with their high expression of SLA II and the costimulatory molecule LFA-1 (46). This capability is shared by DCs (45), although the allogeneic T cell proliferation induced by those cells is frequently stronger than that reported here for 2A10+ monocytes. Altogether, our findings indicate that, although freshly isolated 2A10+ blood cells have phenotypic and morphologic features of monocytes, they can be driven under certain conditions to cells with some characteristics of DCs. Studies on swine DCs isolation and phenotyping will help us to understand their relationship with 2A10+ monocytes.

In summary, we have characterized a new subpopulation of swine monocytes based on the expression of a macrophage differentiation marker, the 2A10 Ag. This subpopulation presents some features of mature tissue macrophages and inflammatory cells. These cells could also be in vitro precursors of DCs, because they share specific morphological and functional properties characteristic of those accessory cells.

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References


