ORIGINAL ARTICLE

Identification of the ectoenzyme CD38 as a marker of committed preadipocytes

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BACKGROUND/OBJECTIVES: Characterisation of the adipocyte cellular lineage is required for a better understanding of white adipose tissue homoeostasis and expansion. Although several studies have focused on the phenotype of the most immature adipocyte progenitors, very few tools exist to identify committed cells. In haematopoiesis, the CD38 ectoenzyme is largely used to delineate various stages of stem cell lineage commitment. We hypothesise that this marker could be used to identify committed preadipocytes.
METHODS: Complementary strategies including flow cytometry, cell-sorting approaches, immunohistochemistry and primary cultures of murine adipose progenitors isolated from different fat pads of control or high-fat diet exposed C57BL/6 J mice were used to determine the molecular expression profile, proliferative and differentiation potentials of adipose progenitors expressing the CD38 molecule.
RESULTS: We demonstrate here that a subpopulation of CD45$^-$ CD31$^-$ CD34$^+$ adipose progenitors express the cell surface protein CD38. Using a cell-sorting approach, we found that native CD45$^-$ CD31$^-$ CD34$^+$ CD38$^+$ (CD38$^+$) adipose cells expressed lower CD34 mRNA and protein levels and higher levels of adipogenic genes such as *Pparg*, *aP2*, *Lpl* and *Cd36* than did the CD45$^-$ CD31$^-$ CD34$^+$ CD38$^-$ (CD38$^-$) population. When cultivated, CD38$^+$ cells displayed reduced proliferative potential, assessed by BrdU incorporation and colony-forming unit assays, and greater adipogenic potential. *In vitro*, both CD38 mRNA and protein levels were increased during adipogenesis and CD38$^-$ cells converted into CD38$^+$ cells when committed to the adipogenic differentiation programme. We also found that obesity development was associated with an increase in the number of CD38$^+$ adipose progenitors, this effect being more pronounced in intra-abdominal than in subcutaneous fat, suggesting a higher rate of adipocyte commitment in visceral depots.
CONCLUSIONS: Together, these data demonstrate that CD38 represents a new marker that identifies committed preadipocytes as CD45$^-$ CD31$^-$ CD34^{low} CD38$^+$ cells.

INTRODUCTION

The resurgence of interest of the scientific community for adipose tissue biology parallels the obesity epidemic and is highlighted by several recent groundbreaking discoveries in the biology of this complex tissue.[1] White adipose tissue (WAT) development can result from both adipocyte hypertrophy (enlargement of existing adipocytes) and adipocyte hyperplasia (increase of adipocyte number owing to the proliferation and/or differentiation of adipose progenitor cells). The extreme plasticity of WAT is also highlighted by its ability to convert into a brown-like adipose tissue, dissipating energy through uncoupling protein-1-mediated thermogenesis.[2,3] In addition to transdifferentiation of mature white into brown-like adipocytes (also called beige or brite adipocytes),[4–7] *de novo* differentiation from pre-existing adipose progenitors[8,9] would be a source of uncoupling protein-1-expressing adipocytes in WAT. Thus, adipose progenitors are key elements for adipose depot formation, maintenance, expansion and plasticity in response to various stimuli.[10] However, much remains unknown about their identity and how they may control adiposity in response to homoeostatic and external cues.

Several observations suggest that different subpopulations of adipose progenitors with independent lineages (adult versus developmental progenitors[11]), differentiation potentials (white versus brown/beige[9,12–14]) and activities could exist inside the same fat pad.[10,15–17] Furthermore, it appears that they may exist in various stages of adipocyte lineage commitment.[18,19] Adipose progenitors, also named adipose-derived stromal cells (ASC), belong to a CD34-positive cell population among the stromal vascular fraction (SVF) of both murine and human WAT, and are negative for haematopoietic (CD45) and endothelial (PECAM1 also named CD31) markers.[20,21] These cells are multipotent and can give rise to osteoblasts, chondrocytes and adipocytes. They also show important paracrine and immunomodulatory activities, which render them attractive for regenerative medicine.[22] Recently, an ASC subset expressing the CD24 antigen has been shown to be capable of generating a functional WAT depot following transplantation into a residual WAT depot of lipodystrophic mice, indicating that the CD24 population contains immature adipose progenitors. Interestingly, CD24 is lost as the cells become committed to adipogenic differentiation.[18,19]

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CD38 expression reflects distinct levels of cell immaturity. The more immature human haematopoietic stem cells (HSC) are isolated according to the CD34^{+} CD38^{−} phenotype, as this subset is highly enriched in multipotent progenitor cells, long-term culture initiating cells and long-term reconstituting stem cells.[23] In addition to being a receptor (CD31 is its main ligand), CD38 is a multifunctional ectoenzyme that catalyses the synthesis and hydrolysis of cyclic ADP-ribose from NAD^{+} to ADP-ribose, and thus constitutes a major regulator of intracellular NAD^{+} and Ca^{2+} levels.[24] Thus, CD38 has been implicated in the regulation of a wide variety of signalling pathways, mainly studied in haematopoietic cells, where it regulates proliferation, activation and migratory processes.[24] Although CD38 has been shown to partly mediate insulin and PPARγ agonist-induced GLUT4 translocation at the plasma membrane of adipocytes,[25] little is known about its role in adipose cell biology. We demonstrate here that a subpopulation of CD45^{−} CD31^{−} CD34^{+} adipose progenitors express the cell surface protein CD38. We found that the CD38^{+} subset, whose percentage is higher in visceral than in subcutaneous fat depots and increases with obesity, was more committed to adipogenesis and less immature than the more proliferative CD38^{−} subset. CD38^{−} cells transformed into CD38^{+} cells during adipocyte differentiation, providing further evidence that CD38 constitutes a new marker for adipogenic commitment.

MATERIALS AND METHODS

Animals
All experiments were carried out in compliance with European Community legislation (2010/63/UE) and approved by the French ethics committee. Experiments were performed on 6–8-week-old male C57BL/6J mice (Harlan, Gannat, France). Animals were housed in a 12-h light/12-h dark cycles at 21 °C with unrestricted access to water and a standard chow diet (UAR) in a pathogen-free animal facility (US006/CREFRE INSERM/UPS) and were killed by cervical dislocation. For high-fat diet (HFD) experiments, 6-week-old mice were exposed to normal-fat diet (NFD; 10% lipids, D12450H Research Diet) or HFD (45% lipids, D12451 Research Diet).

Isolation of SVF cells from murine adipose tissues
SVF of fat pads from 6–8-weeks-old mice were obtained as previously described.[26] Inguinal (ING), epididymal (EPI) and inter-scapular brown adipose tissues (iBAT) were dissected, mechanically dissociated and digested for 30 min at 37 °C with collagenase (collagenase NB 4 Standart Grade from Coger, concentration of 0.4 U/ml diluted in proliferative medium, see below for medium composition) under agitation. After filtration, cells were centrifuged (630 g for 10 min). The pellet defined as SVF was incubated for 5 min in haemolysis buffer (Stem Cell Technologies, Vancouver, BC, Canada) and washed by centrifugation in phosphate-buffered saline (PBS). Cells were counted and used for flow cytometric, cell sorting or culture process.

Primary culture of ASCs and adipocyte differentiation
Following centrifugation, murine SVF cells (isolated from a pool of ING fat pads from five mice per experiment) resuspended in proliferative medium (αMEM plus 0.25 U/ml amphotericin, 100 U/ml penicillin, 100 mg/ml streptomycin, 0.016 mM biotin, 100 μM ascorbic acid, 0.018 mM pantothenic acid and 10% new-born calf serum (NCS)) were plated (10000 cells/cm^2) and rinsed with PBS 3 h after plating. Adherent ASC were grown to confluence in proliferative medium. At confluence, ASC were exposed to an adipogenic cocktail containing 5 μg/ml insulin, 2 ng/ml T3, 33.3 nM dexamethazone, 10 μg/ml transferrin and 1 μM rosiglitazone in complete medium. Cells were cultured at 37 °C (5% CO_2). The medium was changed every 2–3 days all along the culture process. Cells were harvested at the time points indicated in the figure legends.

Flow cytometry and cell sorting
Freshly isolated SVF cells (isolated from fat pads from one mouse per experiment) were stained with 5% new-born calf serum-PBS containing FcR blocking reagent (BD Biosciences, Heidelberg, Germany). Phenotyping was performed by immunostaining with conjugated rat anti-mouse antibodies and compared with isotype-matched control antibodies (Table 1). Cells were washed and analysed on a FACSCanto II or LSR Fortessa flow cytometer (BD Biosciences). Data were acquired with FACSDiva software (BD Biosciences). Data analysis was performed using Kaluza software (Beckman Coulter, Roissy CDG, France).

For ASC cell-sorting experiments, SVF cells from ING fat pads (collected from 20 mice per experiment) were stained with fluorescein isothiocyanate-conjugated CD34, PeCy7-conjugated CD31, PerCP-conjugated CD45 and PE-conjugated CD38 antibodies. Cells negative for CD45 and CD31 were gated, and CD34^{+} CD38^{−} and CD34^{+} CD38^{+} cells were sorted (ARIA FUSION SORP, BD Biosciences). The purity of the enrichments determined by flow cytometry is shown in Supplementary Figure S1.

Bromodeoxyuridine (BrdU) incorporation
Sorted CD45^{−} CD31^{−} CD34^{+} CD38^{−} or CD38^{+} cells were plated in proliferative medium and treated with BrdU (10 μM) for 5 days to get an optimal BrdU incorporation and signal. Cells were then stained using the BrdU Flow kits (BD Pharmingen, San Jose, CA, USA). Cell staining was analysed on a FACSCanto II flow cytometer (BD Biosciences). Data were
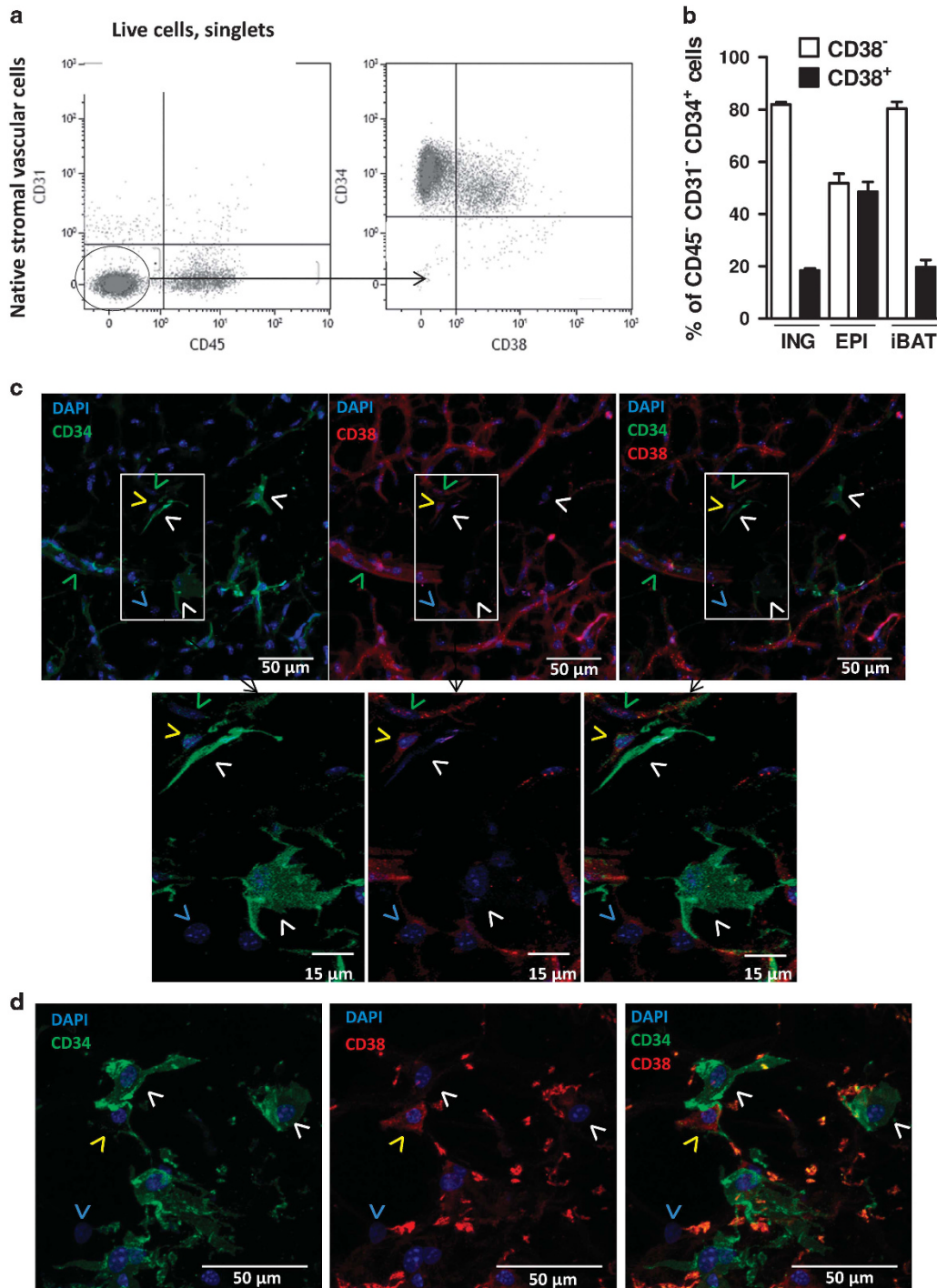
Table 1. Antibodies used for flow cytometry experiments

Name	Clone	Supplier
CD34-FITC	RAM34	BD Biosciences
CD38-PE	90	BD Biosciences
CD45-PerCp	30-F11	BD Biosciences
CD31-PeCy7	390	BD Biosciences

Table 2. Sequences of primers used for quantitative real-time RT-PCR analyses

Target (murine)	Forward	Reverse
36b4	AGTCGGAGGAATCAGATGAGGAT	GGCTGACTTGGTTGCTTTGG
Cd38	GGAGAGCCTACCACGAAGCA	TGTACTCAGTATCTCCTGGCAGTTCT
Cd34	GGAGTTCTGCTGGCCATCTT	ATAAGGGTCTTCACCCAGCC
Cd24	ACGGAGCGGACATGGGCAGA	TTGGTTGCAGTAAATCTGCGTGGGT
Cebpb	GCGCACCGGGTTTCG	CAGGGCCCGGCTGACA
Cebpd	CGACTTCAGCGCCTACATTG	TGAAGAGGTCGGCGAAGAGT
Cebpa	GAGCCGAGATAAAGCCAAACA	GCGCAGGCGGTCATTG
Chop10	GCCAACAGAGGTCACACGC	CCTGGGCCATAGAACTCTGACT
Pparg	AGTGTGAATTACAGCAAATCTCTGTTTT	GCACCATGCTCTGGGTCAA
Lpl	ATCTGCAGAAGGGAAAGGACTC	CTCTTGTTTGTTTGTCCAGTGT
aP2	GATGCCTTTGTGGGAACCTG	GCCATGCCTGCCACTTTC
Cd36	GATGTGGAACCCATAACTGGATTCAC	GGTCCCAGTCTCATTTAGCCACAGTA
Tmem26	ACCCTGTCATCCCACAGAG	TGTTTGGTGGAGTCCTAAGGTC
Cd137	CTCTGTGCTCAAATGGATCAGGAA	TGTGGACATCGGCAGCTACAA

Figure 1. A subset of CD45− CD31− CD34+ cells from murine adipose stromal vascular fraction expresses CD38. (**a**) Flow cytometry was performed on inguinal (ING) SVF to identify CD34+ CD38+ cells in the CD45− CD31− population gated on singlet live cells. Dot plots are representative of 13 independent animals. (**b**) Percentage of CD45− CD31− CD34+ CD38− (white bars) and CD45− CD31− CD34+ CD38+ (black bars) cells in ING (*n* = 13), epididymal (EPI, *n* = 11) and inter-scapular brown adipose depots (iBAT, *n* = 3). (**c**, **d**) Immunostainings of CD34 (green) and CD38 (red) performed on ING sections. Yellow arrow: CD34+ CD38+ cells. White arrow: CD34+ CD38− cells. Blue arrow: CD34− cell. Green arrow: CD34+ CD38+ cells with endothelial phenotype (aligned cells with flattened nuclei). Nuclei are stained blue. Images are representative of three independent experiments.

acquired with FACSDiva software (BD Biosciences) and analysis was performed using the Kaluza software (Beckman Coulter).

Colony-forming unit fibroblast assay. Sorted CD45− CD31− CD34+ CD38− or CD38+ cells were seeded in 25 cm² flasks at a concentration of 16 cells/cm² in proliferative medium as previously described.[27] After 10 days, cells were washed with PBS and dried at room temperature overnight. The colony-forming unit fibroblasts (CFU-f) were stained with RAL stainer

MCDH (Ral Diagnostics, Martillac, France) and scored under an optical microscope. Colonies were defined as clusters of > 50 cells.

RNA extraction and quantitative real-time RT-PCR
Total RNA from either native FACS-sorted or cultivated cells was purified using RNeasy micro-columns. Total RNA of 250–500 ng was reverse-
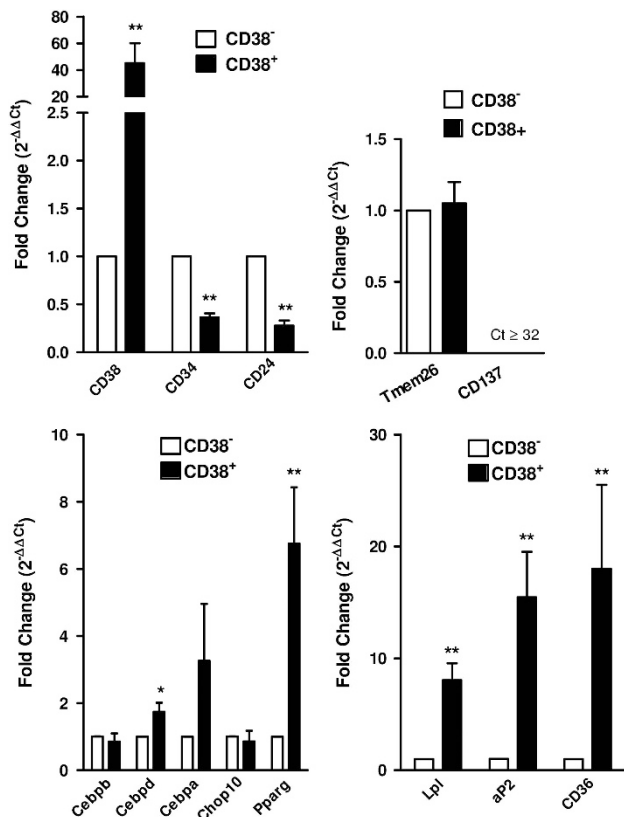
Figure 2. Gene expression in native CD38$^+$ and CD38$^-$ adipogenic progenitors. Native CD45$^-$ CD31$^-$ CD34$^+$ CD38$^-$ and CD45$^-$ CD31$^-$ CD34$^+$ CD38$^+$ subsets were sorted using FACS. Total RNA was then isolated and assayed for mRNA levels of *Cd38*, *Cd34*, *Cd24*, *Tmem26*, *Cd137*, *Cebpb*, *Cebpd*, *Cebpa*, *Chop10*, *Pparg*, *Lpl*, *aP2* and *Cd36* by RT-QPCR. ($n = 5$) *$P < 0.05$ and **$P < 0.01$ compared with CD38$^-$ cells.

transcribed using the High Capacity cDNA Reverse Transcription kit (Applied Biosystem, Waltham, MA, USA), SYBR Green PCR Master Mix (Applied Biosystem), and 0.2 mM primers, on a Viia7TM (Applied Biosystem) instrument. Relative gene expression was determined using the $2^{-\Delta\Delta CT}$ method and normalised to 36B4. The primers are listed in Table 2.

Immunohistochemistry

Adipose tissue sections 300 μm thick (from ING, EPI and iBAT) were incubated in blocking solution (2% normal horse serum and 0.2% triton X-100 in PBS) for 4 h at room temperature and then incubated with primary antibody against CD38 (1:100, Biolegend, San Diego, CA, USA) and CD34 (1:250, Abcam, Cambridge, UK) for 24 h at room temperature. After several rinses in PBS-0.2% triton X-100, sections were incubated overnight at 4 °C with Alexa 488 conjugated anti-rabbit and Alexa Fluor 594 conjugated anti-rat secondary antibodies (Life Technologies, Carlsbad, CA, USA). Nuclei were stained with 4',6-diamidino-2-phenylindole (Sigma Aldrich, St Louis, MO, USA) and sections were mounted on a coverslip. Imaging was performed using a confocal Laser Scanning microscope (LSM 780, Carl Zeiss, Oberkochen, Germany). Image analysis was performed using Fiji software (NIH, Bethesda, MA, USA).

Statistical analysis

The number of independent experiments are detailed in each Figure Legend. Experiments done previously were used to determine sample size with adequate statistical power. No animals or data were excluded. Data collection and analysis were not randomised but performed blind to investigators. All data are expressed as mean ± s.e.m. A Mann–Whitney statistical test (two-tailed) was used to calculate final *P*-values using GraphPad Prism software. Differences among groups were considered significant at $P < 0.05$.

RESULTS

A subset of CD45$^-$ CD31$^-$ CD34$^+$ cells from murine adipose SVF expresses CD38

Multi-parameter flow cytometry analyses were performed on native cells from the SVF of murine white (subcutaneous ING and visceral EPI) and brown (iBAT) fat pads using anti-CD45, -CD34, -CD31 and -CD38 antibodies. We found that the CD45$^-$ CD31$^-$ CD34$^+$ adipose progenitor population was heterogeneous regarding the expression of the CD38 molecule (Figure 1a). Although CD38$^+$ cells made up < 20% of CD45$^-$ CD31$^-$ CD34$^+$ adipose progenitors in both ING and iBAT depots (Figure 1b), this percentage reached ~ 49% in the murine EPI fat pad (Figure 1b). Immunohistochemistry experiments performed on murine ING fat sections confirmed the existence of fibroblastic CD34$^+$ cells expressing the CD38 antigen (Figures 1c and d, yellow arrows). It is noteworthy that CD38$^+$ cells expressed CD34 at lower levels compared to the CD38$^-$ population, as shown both by flow cytometry (CD34 mean fluorescent intensity for the CD38$^-$ subset: 10.3 ± 0.7; CD34 mean fluorescent intensity for the CD38$^+$ subset: 5 ± 0.3, Figure 1a) and immunohistochemistry experiments (Figures 1c and d, yellow and white arrows). CD34^{low} CD38^{high} cells were also present in the EPI and iBAT tissue sections (yellow arrow, Supplementary Figure S2). Mature adipocytes did not express CD38, unlike endothelial cells, which were strongly positive for CD38 (Figure 1c, green arrows and Supplementary Figure S3), and a fraction of haematopoietic cells (Supplementary Figure S3).

CD38$^+$ adipose progenitors are more committed to adipogenesis than the more immature and proliferative CD38$^-$ subpopulation

In order to characterise CD45$^-$ CD31$^-$ CD34$^+$ CD38$^-$ and CD38$^+$ subpopulations, a cell-sorting approach using FACS was used (the purity of the sorted populations is shown in Supplementary Figure S1). RT-QPCR analyses showed, as expected, enrichment in *Cd38* mRNA levels in the CD38$^+$ compared with the CD38$^-$ subset (Figure 2). The native CD38$^+$ population also displayed lower *Cd34* expression than the CD38$^-$ subset (Figure 2), further confirming the flow cytometry and immunohistochemistry findings (Figures 1a,c and d). The expression of *Cd24*, which is expressed by the more immature adipose progenitors,[19] was also significantly lower in the CD38$^+$ subset, suggesting that these cells might be less immature than their CD38$^-$ counterparts. No difference was found in the gene expression of several members of the CAAT/enhancer binding protein family including *Cebpb*, *Cebpa* or the dominant-negative isoform *Chop10* and of specific markers of brite progenitors such as *Tmem26* and *Cd137*[28] (of note *Cd137* was expressed at very low level in each cell subset). However, expression of transcription factors such as *Cebpd* and *Pparg2* as well as several markers of adipocyte differentiation including *Lpl*, *aP2* and *Cd36* were greatly enhanced in the native CD38$^+$ subset (Figure 2). The adipogenic potential of the sorted CD38$^-$ and CD38$^+$ populations was next analysed *in vitro* after induction of adipogenic differentiation. The vast majority of CD38$^+$ cells had accumulated lipid droplets 4 days after the addition of the adipogenic cocktail, whereas few adipocytes were detected in the CD38$^-$ subset at that time (Figure 3a, left panel). RT-QPCR analyses revealed higher expression of *aP2*, *Lpl* and *Cd36* in the CD38$^+$ subpopulation (Figure 3b, right panel). Note that the CD38$^-$ subset was able to reach levels of differentiation and adipogenic gene expression similar to those of CD38$^+$ cells but after a longer exposure time in the adipogenic cocktail (that is, 7 days, see Supplementary Figure S4), suggesting that CD38$^-$ cells were delayed in the adipogenic programme. To investigate the proliferative capacity of the two subpopulations, sorted cells were plated and assessed for BrdU incorporation and for their ability to give rise to colony-forming unit fibroblasts. Figures 3b and c show that the CD38$^+$ subset displayed lower BrdU incorporation and
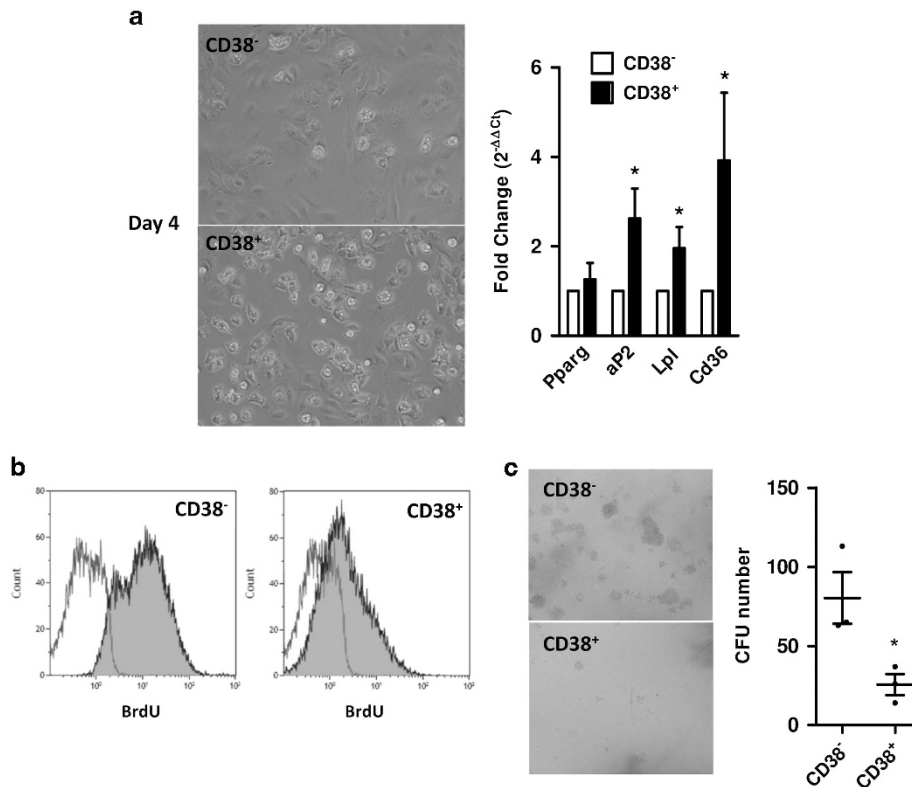
Figure 3. Sorted CD38$^+$ subset is more committed to adipogenesis compared to the more immature and proliferative CD38$^-$ subpopulation. (**a**) Sorted CD45$^-$ CD31$^-$ CD34$^+$ CD38$^-$ and CD45$^-$ CD31$^-$ CD34$^+$ CD38$^+$ subsets were plated in adipogenic medium. four days later, lipid accumulation was seen in almost all CD38$^+$ cells. Total RNA was isolated and assayed for mRNA levels of *Pparg*, *aP2*, *Lpl* and *Cd36* by RT-QPCR. (*n* = 4) (**b**) Sorted CD45$^-$ CD31$^-$ CD34$^+$ CD38$^-$ and CD45$^-$ CD31$^-$ CD34$^+$ CD38$^+$ subsets were plated in proliferative medium and incubated with BrdU. Five days later, cells were analysed for BrdU incorporation by flow cytometry. Histograms are representative of three independent experiments. (*n* = 3). (**c**) Sorted CD45$^-$ CD31$^-$ CD34$^+$ CD38$^-$ and CD45$^-$ CD31$^-$ CD34$^+$ CD38$^+$ subsets were plated in proliferative medium (16 cells/cm^2 in a 25 cm^2 flask). Ten days later, CFU were counted. (*n* = 3) *$P < 0.05$ compared with CD38$^-$ cells.

gave rise to fewer colony-forming unit fibroblasts than the more proliferative CD38$^-$ subpopulation. Altogether, these findings demonstrate that native CD38$^+$ cells are less immature and more committed to adipogenesis compared with the more proliferative and immature CD38$^-$ subset.

CD38 expression increases with adipocyte differentiation

To further study the link between CD38 and adipogenesis, the whole SVF isolated from murine ING fat pad was cultivated and induced to differentiate into adipocytes once cells reached confluence (d0). As shown in Figure 4a, increased expression of the adipogenic markers *Pparg2* and *aP2* was associated with higher *Cd38* mRNA levels 4 and 7 days after induction of differentiation than in undifferentiated cells (d0). Flow cytometry experiments performed in the same conditions showed a large increase in CD38 protein content along with adipogenesis (Figure 4b). Because CD38$^-$ cells seemed delayed in the adipogenic programme and because CD38 expression increased during adipogenesis, we then wondered whether CD38$^-$ cells became CD38$^+$ when committed to adipocyte differentiation. We found that cultivating CD38$^-$ sorted cells in the adipogenic medium converted them into CD38$^+$ cells, as we detected 19.5 ± 5% and 64.6 ± 8% of CD38$^+$ cells in the CD38$^-$ cultivated cells after 2 and 5 days, respectively, of culture in adipogenic conditions (Figure 4c). *In vivo*, we next assessed whether adipose tissue expansion was associated with concomitant changes in CD38-expressing adipose progenitors. Mice exposed to a HFD for 8 or 12 weeks showed significant increases in body weight and

white fat pads weights (Supplementary Figure S5). As shown in Figure 4d, while no difference was observed after 8 weeks of HFD, the number of CD45$^-$ CD31$^-$ CD34$^+$ CD38$^+$ cells in the ING fat pads was increased in mice submitted for 12 weeks to HFD compared with control mice (NFD). The increase of CD38$^+$ cells was faster and more pronounced in the visceral fat pads, as it was already observed after 8 weeks of HFD (Figure 4d). The same trend was observed for the CD38$^-$ cell subset (Supplementary Figure S6).

DISCUSSION

The identification and characterisation of adipose progenitors is of crucial interest for our understanding of the cellular and molecular mechanisms regulating adipogenesis. Here, we have identified committed preadipocytes as CD45$^-$ CD31$^-$ CD34^{low} CD38$^+$ cells (Figure 4e). Berry *et al.*[18,29] recently proposed a model for adipogenesis where loss of CD24 expression was associated with the transition from immature progenitors (that did not express *Pparg2*) to preadipocytes (that expressed *Pparg2*). Our analysis of CD45$^-$ CD31$^-$ CD34$^+$ native cells shows that, compared with CD38$^-$ cells, the CD38$^+$ subset expressed low *Cd24* and high *Pparg2* levels (as well as additional adipogenic genes), indicating that CD38 is expressed by cells less immature and more committed to adipogenesis. This is supported by the low proliferative capacity and high adipogenic properties of CD38$^+$ cells. RT-QPCR, flow cytometry and *in situ* immunofluorescence experiments also showed that the CD38$^+$ subset displayed low expression of CD34, known to be lost when preadipocytes
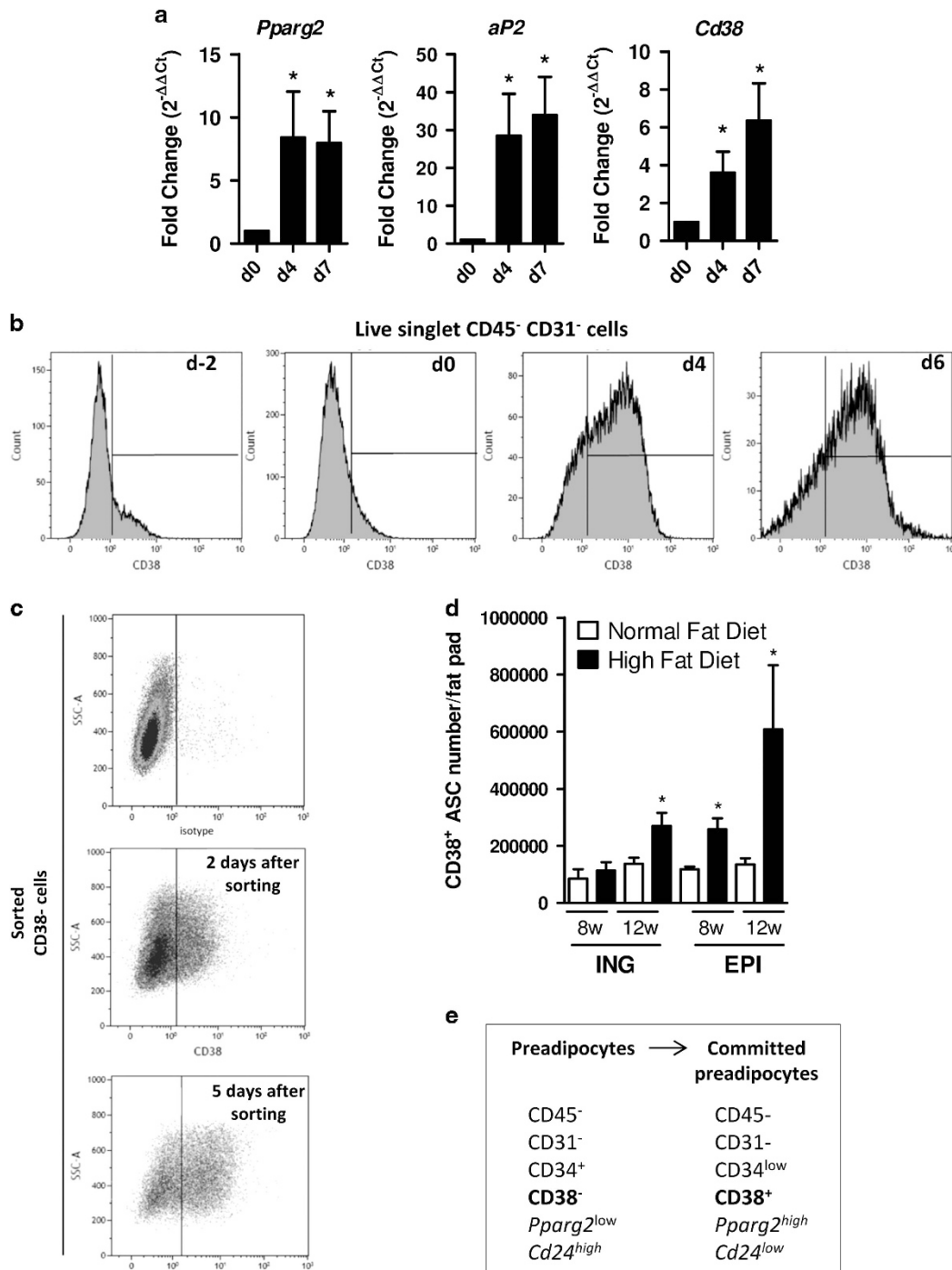
Figure 4. CD38 expression increases during adipogenesis. (**a**) SVF cells from murine inguinal fat pad were plated in proliferative medium, grown to confluence and either lysed (day 0) or induced to differentiate into adipocytes and lysed at day 4 or day 7. Total RNA was then isolated and assayed for mRNA levels of *Pparg, aP2* and *Cd38* by QPCR. n = 3 *P < 0.05 compared with day 0. (**b**) SVF cells from murine inguinal fat pad were plated in proliferative medium and harvested 2 days before confluence (d-2), at confluence (d0) and 4 or 6 days after induction of adipocyte differentiation. CD38 expression was analysed by flow cytometry. Histograms are representative of three independent experiments. (n = 3). (**c**) Sorted CD38⁻ cells were plated in adipogenic medium. Two and 5 days later, cells were harvested and analysed for CD38 expression by flow cytometry experiments. Dot plots are representative of three independent experiments. (**d**) Quantification by flow cytometry of CD45⁻ CD31⁻ CD34⁺ CD38⁺ cell number in the whole SVF of inguinal and epididymal fat pads of mice submitted to normal-fat diet (NFD, 10% lipids) or to high-fat diet (HFD, 45% lipids). (n = 4–5 mice per group) *P < 0.05 compared with NFD. (**e**) Schematic representation of the adipocyte cellular lineage. Our findings demonstrate that CD38 expression delineates a subpopulation of cells that are committed to differentiation.

differentiate into mature adipocytes.[29] This further reinforces our conclusion that CD38 is expressed by cells committed to the adipogenic programme. The resistance of CD38 knock-out mice to diet-induced obesity is fully consistent with this conclusion.[30]

Interestingly, CD38⁻ cells become CD38⁺ during the culture process. Such a fluctuation in stem cells markers expression has been already shown including for Sca-1.[31] Further investigations will be needed to demonstrate whether CD38 is a marker for

multilineage commitment of ASC, as demonstrated for bone marrow progenitor cells[32] and whether it is also expressed by ASC committed into brite adipogenesis or into the chondrocyte and osteoblast differentiation pathways.

Although more and more evidence suggest that obesity and associated abnormal glucose and lipid metabolism are associated with modifications of the NADH/NAD$^+$ system,[33,34] the specific molecular components of this pathway involved in diet-induced obesity are not entirely deciphered. However and consistently with our conclusions, high human *CD38* expression has been found in adipose tissues of obese/type 2 diabetes subjects.[35] Our data revealed a higher percentage of CD38$^+$ adipose progenitors in the EPI WAT than in the ING one in steady state, as well as a faster and higher increase in CD38$^+$ subset in the EPI pad in HFD fed mice. Because the same trend was observed for CD38$^-$ cells, we propose that this proliferative cell subset might be recruited and committed into CD38$^+$ cells during the development of adipose tissues in such adipogenic conditions. These results are consistent with the adipocyte hyperplasia phenomenon that was shown to be depot specific and much more pronounced in intra-abdominal than in subcutaneous WAT during obesity develop-ment in mice.[36] Given that CD38 defines committed preadipo-cytes, one can reasonably postulate that higher CD38 expression reflects a higher rate of adipogenesis in visceral versus sub-cutaneous adipose tissues.

This study reveals CD38 as a new marker of commitment into adipocyte differentiation. Analysis of CD38 expression could thus help to detect the appearance of hyperplasia and therefore might provide an index for adipose tissue expandability, a key determinant in protection against the metabolic syndrome associated with obesity.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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