A subset of dendritic cells express joining chain (J-chain) protein

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Summary

Joining chain (J-chain) is well known as an integrated component of dimeric immunoglobulin A (IgA) and pentameric IgM. We show here that the J-chain protein is also expressed in a subset of CD11c$^+$ dendritic cells (DC) in C57BL/6 mice. J-chain knockout mice (J$^{-/-}$ mice) had a reduced fraction of CD4$^-$/CD8α^+ and mPDCA-1$^+$ DC in the spleen. J$^{-/-}$ mice also had reduced levels of RNA for the immunoregulatory enzyme indoleamine 2,3-dioxygenase (IDO) in the spleen. Furthermore, in lymph nodes from C57BL/6 mice the majority of J-chain-expressing CD11c$^+$ cells also expressed IDO, while the number of IDO-expressing cells in lymph nodes and the amount of IDO protein in splenic CD11c$^+$ cells were reduced in J$^{-/-}$ mice. Also, J$^{-/-}$ mice had a lower ratio of kynurenine/tryptophan in serum compared to C57BL/6 mice, indicating a lower overall IDO activity in J$^{-/-}$ mice. We also show that J$^{-/-}$ mice are less susceptible to tolerance induction than C57BL/6 mice. In conclusion, our data show that J-chain protein is expressed outside the B-cell compartment in a subset of immunoregulatory DC that are compromised in animals that cannot express J-chain.

Keywords: dendritic cells; indoleamine 2,3-dioxygenase; joining chain; T cells

Introduction

The transport of immunoglobulin A (IgA) over mucosal membranes is mediated by the ability of its dimeric form to interact with the polymeric immunoglobulin (poly-Ig) receptor on the basolateral side of secretory epithelial cells.[1] After binding to this receptor, dimeric IgA is transcytosed and released on the apical surface.[2] The binding to the poly-Ig receptor is dependent on the presence of joining chain (J-chain).[3] As a consequence, J-chain-containing IgM can be transcytosed on to mucosal membranes.[4] J-chain expression has been described as being confined to the B-cell lymphoid compartment. More specifically, it was initially claimed that only plasma cells expressed J-chain and that all plasma cells did so regardless of the immunoglobulin isotype expressed.[5] However, it has been shown in humans that a subset of plasma cells do not express J-chain,[6,7] and this has been confirmed in mice.[8] Using J-chain knockout (J$^{-/-}$) mice we recently showed that these animals have a compromised B-cell memory.[9] By adoptive transfer experiments it was shown that this phenotype was transferred by primed T cells from J$^{-/-}$ mice rather than primed B cells, which pointed to a possible function for the J-chain outside the B-cell compartment.

It is therefore interesting to note that J-chain RNA expression has been described in human dendritic cells (DC).[10] Interestingly, J-chain expression was higher in plasmacytoid DC (pDC) than in monocyte-derived DC.[10] The DC are the central cell population involved in antigen presentation and the initiation of immune responses.[11–13] They can be subdivided into several distinct subsets that have complex developmental pathways and in which individual cells may transit between defined populations by acquisition of new surface markers.[13] It has also been shown that DC are involved in the qualitative outcome of immune interactions. Thus, certain DC can deliver a tolerogenic rather than an immunogenic signal to the T cell to which it presents the relevant antigen.[14] One important mediator of a tolerogenic signal has been shown to be the enzyme indoleamine 2,3-dioxygenase (IDO), which breaks down the essential amino acid tryptophan to kynurenine and has immunoregulatory properties.[15,16] IDO is expressed in a subset of CD8α^+ DC and in pDC.[17,18] In this paper we show that a population of DC expresses the J-chain protein and that a major fraction of these DC coexpress IDO. Consequently, J$^{-/-}$ mice have a decreased number of CD4$^-$/CD8α^+ and mPDCA-1$^+$ DC, and a perturbed capacity for tolerance induction.

Materials and methods

Mice, bone-marrow chimeras and induction of tolerance

Age-matched 8- to 12-week-old C57BL/6, B6.SJL and $J^{-/-}$ mice were kept in the animal facility at Lund University. All animal experiments were performed in accordance with approved protocols from the local animal ethics committee. For the creation of bone marrow chimeras 10^7 bone marrow cells from 8- to 10-week-old female $J^{-/-}$ mice and 8-to 10-week-old female SJL mice were mixed and injected into sex-matched irradiated (900 rads) C57BL/6 hosts and analysed 70 days later using fluorescence-activated cell sorting (FACS). To induce oral tolerance mice were fed 50 mg ovalbumin (OVA; Fraction VI; Sigma, St Louis, MO) dissolved in phosphate-buffered saline (PBS), or PBS alone as a control, via a gastric tube with a steel feeding needle (Allgaier Instrument, Frittlingen, Germany), and systemic tolerance was induced by injecting 5 mg OVA intravenously. Seven days after immunization the animals were injected with 100 μg OVA precipitated with alum and OVA-specific immunoglobulin serum levels were determined 14 days later using enzyme-linked immunosorbent assay with 5 μg/ml OVA as the coating antigen and a rabbit anti-mouse horseradish peroxidase (HRP) conjugated secondary antibody (Dako AS, Glostrup, Denmark) to determine the levels of OVA-specific IgG. As a substrate 3,3′,5,5′-tetramethylbenzidine (TMB) was used (Sigma-Aldrich). The titre was defined as the dilution corresponding to 50% of maximum absorbance. For quantification of T-cell proliferation T cells were purified from spleens using Mouse pan T Dynabeads (Dynal, Oslo, Norway) according to the manufacturer's protocol. T cells were cultured at 5×10^5 cells/well together with 5×10^5 irradiated, syngeneic spleen cells (3000 rads) with or without OVA in 0·2 ml RPMI (Invitrogen, Carlsbad, CA) supplemented with 7·5% fetal calf serum (Sigma). Proliferation was quantified after 72 hr by adding 1 μCi/well [^{3}H]thymidine during 16 hr.

Flow cytometric analysis and immunofluorescence

Spleens from the indicated mouse strains were digested with Collagenase D (Roche Diagnostics, Basel, Switzerland) at 37° for 30 min before the preparation of single cell suspensions and FACS analysis. The following antibodies were used for staining: CD45.2-fluorescein isothiocyanate (FITC), CD45.1-phycoerythrin (PE), CD8α-peridinin chlorophyll protein (PerCP), CD4-allophycocyanin (APC), CD11c-biotin/FITC, LY-6C-biotin (BD, Franklin Lakes, NJ), B220-cyanine 5 (CY5) (RA3.6B2) and mPDCA1-PE (Miltenyi Biotec, Gladbach, Germany). Analysis was performed on a FACS Calibur (BD). Freshly isolated spleens were made into single cell suspensions and cultured at 2×10^5 cells/well in 96-well tissue culture plates in 200 μl RPMI-1640 with 25 μg/ml lipopolysaccharide (BD) for 3 days and were thereafter spun on to glass slides. Lymph nodes were embedded in OCT compound (Tissue-Tek®; Sakura Finetek, Torrance, CA), and snap-frozen in liquid nitrogen. Cryosections (5–6 μm) were prepared on microscope slides, air-dried and frozen at −20° until the staining procedures were performed. Acetone-fixed sections were incubated with blocking 1% bovine serum albumin, 10% serum and FcRII/III blocker solution followed by Avidin/Biotin Blocking kit (Vector, Burlingame, CA). Thereafter incubation with biotinylated primary antibodies against Igκ (187.1) and IgL (Southern Biotechnology Associates, Inc., Birmingham, AL), anti-J-chain labelled with Alexa, Fluor® 647, anti-CD11c (BD PharMingen), or the appropriate isotype controls (mouse IgG1 and Armenian hamster IgG), was performed on fixed samples for 30 min at room temperature, followed by streptavidin Alexa Fluor® 488-conjugated secondary antibodies (Invitrogen) and anti-Armenian hamster CY3 (Jackson Immuno Research, West Baltimore, PA). Other sections were stained with anti-CD11c-FITC and biotinylated anti-J-chain followed by streptavidin-conjugated-HRP and Alexa Fluor® 568 tyramide (Miltenyi Biotec). Staining for IDO was performed using an anti-mouse IDO antibody (Chemicon International, Temecula, CA) and an M.O.M kit from Vector following the manufacturer's instructions. The slides were mounted using VectaShield mounting medium (Vector) and inspected under a Zeiss microscope (Burlingame, CA). The anti-J-chain monoclonal antibody was made by immunizing $J^{-/-}$ mice with purified human J-chain.[6,19] The established hybridomas were screened for the immunizing antigen and secondarily screened for reactivity to recombinant, mouse J-chain expressed in insect cells. The selected hybridoma (4/5) was expanded and antibody-purified using conventional methods.

Quantitative polymerase chain reaction (qPCR) analysis

For the qPCR analyses anti-CD11c magnetic beads (Miltenyi Biotec) were used to isolate the indicated spleen cell populations. Subsequently, RNA was prepared using a High Pure RNA Isolation kit (Roche, Mannheim, Germany), while complementary DNA (cDNA) synthesis and real-time quantitative PCR (qRT-PCR) were performed using Platium® SYBR® Green Q-PCR SuperMIX-UDG (Invitrogen) according to the manufacturer's instructions. The cDNA was diluted in five-fold increments and used for PCR amplifications. The qRT-PCR was performed with the following primers: J sense (5′-ATGAGGACCCACCTGCT TCTCTGG-3′), anti-sense (5′-AGGGTAGCAAGAATCGG GGGTCAA-3′); β_2-microglobulin sense (5′-CACCCGCCT CACATTGAAAT-3′), anti-sense (5′-TCGATCCCAGTAGA

CGGTCTT-3′); IDO sense (5′-AAGTTGGGCCTGCCTCC TATTC-3′), anti-sense (5′-TGTCGTGCAGTGCCTTTTCC AAT-3′); glyceraldehyde 3-phosphate dehydrogenase (GADPH) sense (5′-CCTGCACCACCAACTGCTTA-3′), anti-sense (5′-TCATACTTGGCAGGTTTCTCCA-3′). Samples were separated on agarose gels and analysed for primer dimers. The qRT-PCR experiments were performed in triplicate. The threshold cycle (Ct) for the endogenous control messenger RNA (mRNA) and the target signals was determined, and relative RNA quantification was calculated. For the RT-PCR shown in Fig. 1(a) the same primers were used without qPCR labelling. The RT-PCR from RAG mice were blotted onto nylon filters, probed with radiolabelled J-chain probe, washed at high stringency conditions and exposed on a phosphoimager plate.

Western blotting

Sera were collected from wild-type (wt) and $J^{-/-}$ mice and electrophoresis was performed using a 12%, denaturating polyacrylamide gel. Equal amounts of serum (0·5 μl) were loaded in each lane. The protein was transferred to a nylon membrane (Hybond-C extra; Amersham Pharmacia Biotech, Uppsala, Sweden) using a Trans-blot SD system (Bio-Rad, Laboratories). The filters were blocked overnight in 5% dry fat-free milk in PBS-Tween (PBS-T), followed by incubation with a biotinylated anti-J-chain monoclonal antibody (4/5), anti-IDO (see above) or rabbit anti-actin (US Biologicals, Swampscott, MA). HRP-conjugated streptavidin (Southern Biotechnology Associates, Inc.), anti-mouse HRP or goat anti-rabbit HRP (Molecular Probes) was thereafter added, and the filters were developed using ECL-reagent (Amersham Pharmacia Biotech) according to the manufacturer's protocol.

Determination of serum kynurenine and tryptophan levels

Sera were collected from age- and sex-matched wt and $J^{-/-}$ mice and analysed simultaneously for kynurenine and tryptophan on a high-performance liquid chromatograph (HPLC; Hewlett-Packard Series 1100; Hewlett-Packard, Santa Clara, CA) with an XTerra^{TM} MSC_{18} column (Watge, Milford, MA), as described previously.[20] Peak hight values for kynurenin and tryptophan were determined and the ratios were calculated.

Results

Mouse CD11c$^+$ cells express J-chain RNA

We initially wanted to verify in the mouse system, the finding from human gene array studies that J-chain RNA

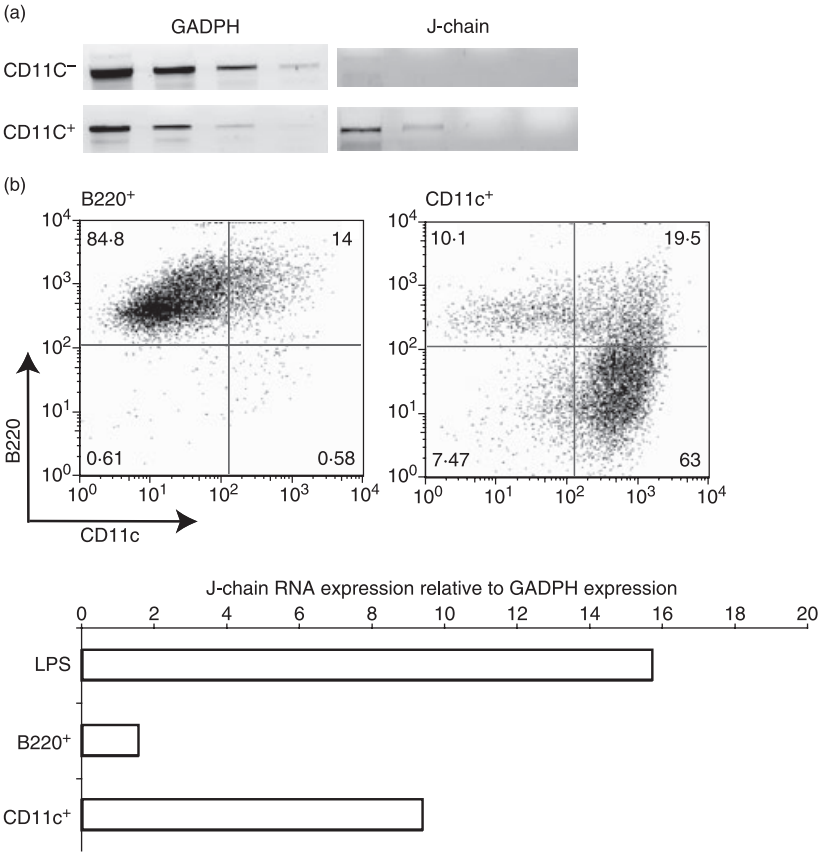

Figure 1. J-chain expression in CD11c$^+$ cells. (a) $RAG^{-/-}$ spleen cells were fractionated into CD11c$^+$ (90% CD11c$^+$) and CD11c$^-$ populations using magnetic beads, RNA prepared and used for cDNA synthesis followed by PCR amplification. Southern blots of RT-PCR products obtained with J-chain or GADPH primers (see Materials and methods) in serial, two-fold dilutions of PCR product is shown. (b) C57BL/6 spleen cells were positively selected using magnetic beads into CD11c$^+$ and B220$^+$ cells, respectively. On the top of the panel the dot-plots obtained in the FACS analysis of the enriched populations are shown. RNA and cDNA were prepared from these cells and analysed by qRT-PCR and the data are shown in the lower part of the figure, data from the J-chain RNA expression in the different cell populations are shown in relation to GADPH. The level of J-chain RNA expression is also compared to that seen in B cells stimulated *in vitro* with LPS for 72 hr, as indicated. All assays were performed in triplicate and the variability was in all cases < 5%.

was expressed in pDC or other DC.[10] To this end, we separated spleen cells from RAG$^{-/-}$ mice using anti-CD11c-coated magnetic beads into a CD11c^{+} and a CD11c^{-} fraction. It should be pointed out that the CD11c^{+} population thus obtained was only 80–90% CD11c^{+}. However, because mouse pDC are CD11c^{dull},[21,22] a more stringent enrichment based on this marker could result in the loss of the cell population of interest. As shown in Fig. 1(a), J -chain RNA expression could be detected in the CD11c^{+} fraction from RAG$^{-/-}$ spleen cells using RT-PCR.

These data are in agreement with previous observations in the human system.[10]

To investigate whether CD11c^{+} cells from immunocompetent mice also expressed J-chain, we used the same CD11c enrichment procedure as above on spleens from C57BL/6 mice. Furthermore, we also enriched for B cells using B220-coated magnetic beads and prepared stimulated B cells by activating splenic B cells for 72 hr with LPS. From all these cells, RNA was prepared and analysed for J-chain expression using qRT-PCR with GADPH expression as reference (Fig. 1b). The data showed that J-chain RNA expression was upregulated in B cells after LPS stimulation and the expression of J-chain RNA in the CD11c-enriched cell fraction was 60% of that seen in LPS-stimulated B cells, but at least four times higher than that observed in the resting B-cell population. Thus, even if the cell populations used were not homogeneous, the J-chain RNA expression seen in the CD11c-enriched population cannot be explained by B-cell contamination. We concluded from these experiments that CD11c-enriched cells expressed J-chain RNA.

A fraction of mouse CD11c^{+} cells express J-chain protein

We next wanted to investigate whether CD11c^{+} cells also expressed J-chain at the protein level. To this end we used a monoclonal antibody to mouse J-chain by immunizing J$^{-/-}$ mice with purified J-chain (see ref. 19 and Materials and methods). This antibody is specific for J-chain in Western blot analysis of serum from C57BL/6 mice using serum from J$^{-/-}$ mice as control (Fig. 2a). We also prepared whole cell extracts from LPS-stimulated B cells, B220-enriched resting B cells, CD11c^{-} cells, spleen cells and CD11c^{+} cells and analysed for J-chain expression. Although we could only detect very low levels of J-chain expression in whole cell extracts, it could still be seen that J-chain protein was only expressed in extracts from LPS-stimulated B cells and CD11c^{+} cells using this method (Fig. 2b). We also investigated the specificity of the anti-J-chain antibody by immunostaining and could show that only LPS-activated B cells from wt C57BL/6 mice, but not from J$^{-/-}$, mice were labelled (Fig. 2c). We then continued to investigate J-chain protein expression in lymph nodes from C57BL/6 mice. As shown in Fig. 2(d), CD11c^{+} cells were seen both within and outside B-cell follicles delineated by the anti-IgL staining. As expected, anti-J-chain antibody stained cells within the B-cell follicles and also cells that were anti-IgL^{+} outside the follicles with a plasma cell appearance. However, J^{+} cells could be observed outside B-cell follicles that were also positive for CD11c. We concluded from these experiments that a fraction of CD11c^{+} cells *in situ* express the J-chain protein.

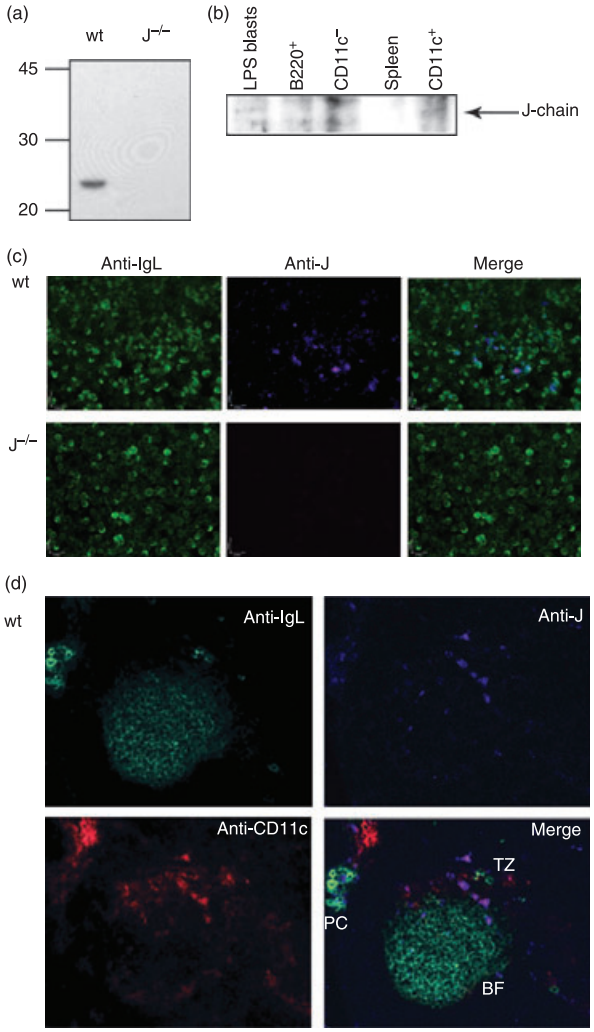

Figure 2. J-chain protein is expressed in CD11c^{+} cells. (a) Western blot using the anti-J-chain antibody (4/5; see Materials and methods) that stained a band in serum from C57BL/6 mice that is absent in serum from J$^{-/-}$ mice. (b) Whole cell protein extracts from the indicated cell populations were analysed for J-chain expression using the anti-J-chain monoclonal antibody. (c) Cytospins prepared from LPS-stimulated B cells from C57BL/6 and J$^{-/-}$ mice illustrating the specificity of the anti-J-chain antibody in immunohistology after staining with anti-IgL (green) and anti-J-chain (purple). (d) Frozen sections were prepared from lymph nodes from C57BL/6 mice and stained with anti-IgL (green), anti-J-chain (purple) and anti-CD11c (red), as indicated. In the panel showing the merged image plasma cells (PC), a B-cell follicle (BF) and the T-cell zone (TZ) are indicated.

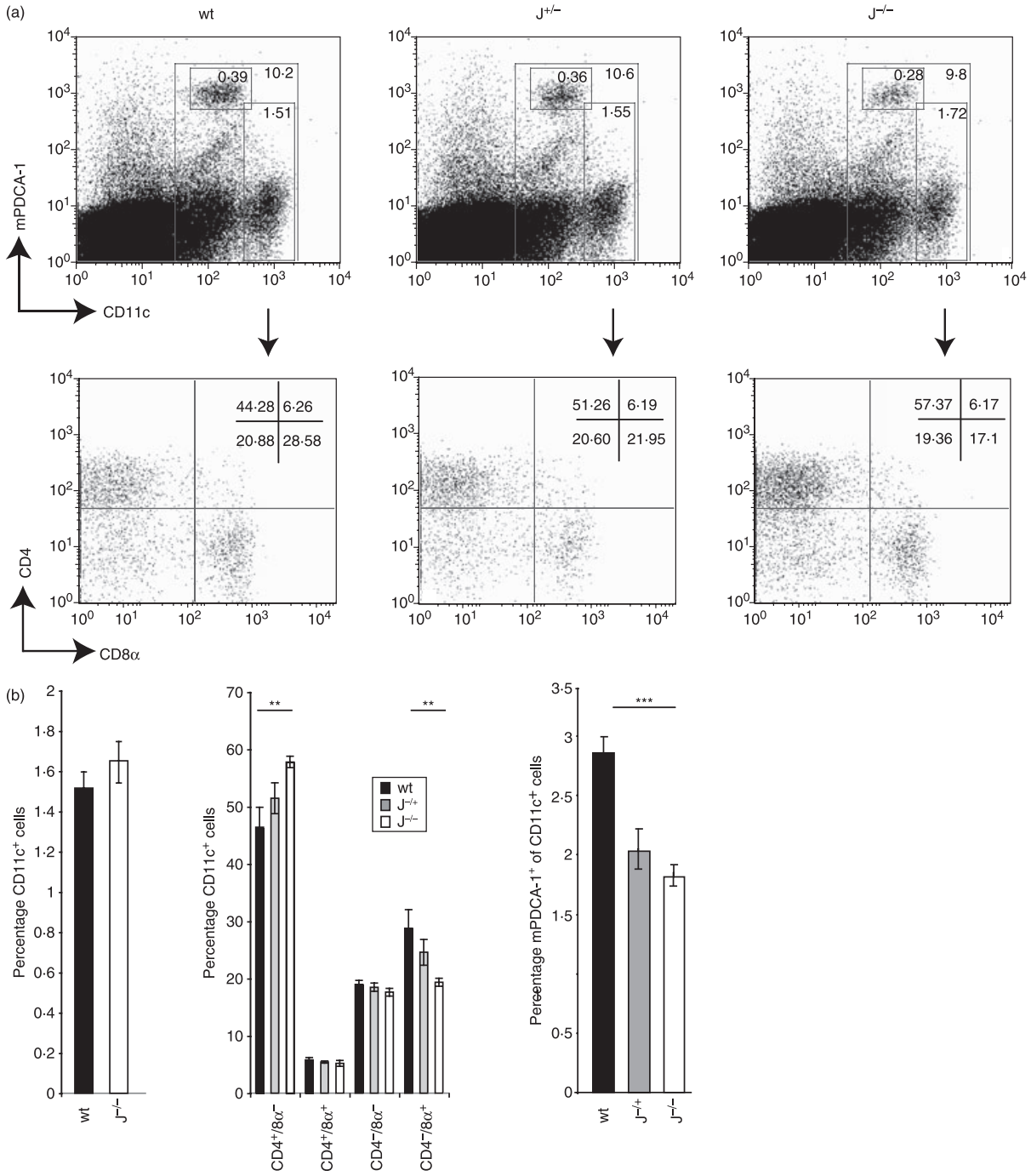

Figure 3. Abnormal distribution of DC in $J^{-/-}$ compared to C57BL/6 mice as revealed by FACS analysis. (a) Dot-plots are shown from a typical experiment to illustrate the gating used to quantify the different subsets of DC in C57BL/6, $J^{+/-}$ mice and $J^{-/-}$ mice, respectively. The numbers in the upper plots represent the percentage of all cells in the lymphocyte gate while the numbers in the lower plots represent the percentage of cells in the CD11c gate. (b) Bar graphs representing data from 10 animals of each genotype (C57BL/6, $J^{+/-}$ and $J^{-/-}$ mice) are shown for the percentage of total $CD11c^+$ cells, and the various subpopulations of $CD11c^+$ cells, as indicated. The error bars represent the standard deviation. Statistical analysis was performed using Student's *t*-test; $^{***}P < 0{\cdot}001$, $^{**}P < 0{\cdot}01$and $^{*}P < 0{\cdot}05$.

$J^{-/-}$ mice have a changed distribution of DC subpopulations

Having determined that J-chain RNA expression could be detected also in mouse DC and extended this observation to the protein level, we next wanted to investigate whether J-chain expression had an impact on DC development or function. Thus, we first analysed spleens from C57BL/6 mice or $J^{-/-}$ mice for the representation of various DC populations. We also included $J^{+/-}$ mice in this

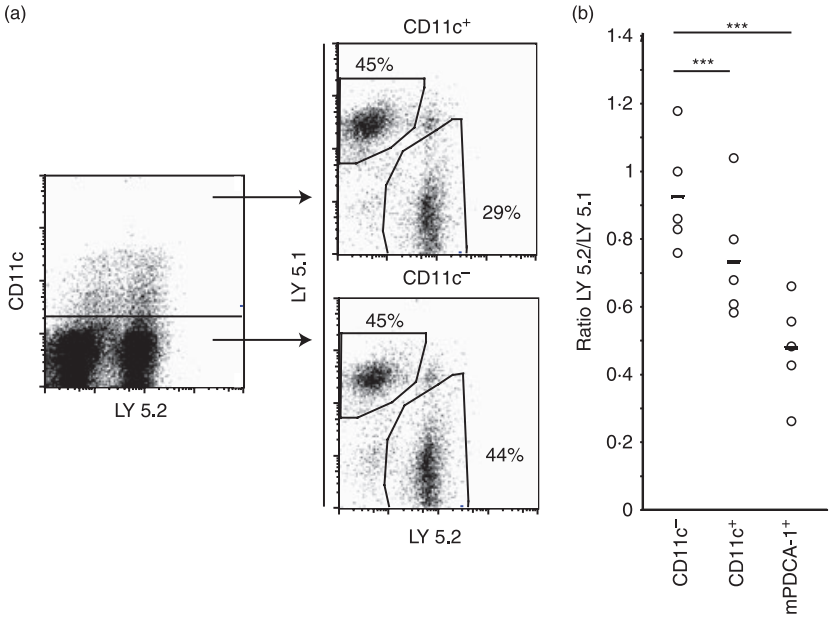

Figure 4. The mPDCA-1 defect in $J^{-/-}$ mice is intrinsic to the haematopoietic compartment. Bone marrow chimeras were constructed with 50% cells from B6.SJL mice and 50% cells from $J^{-/-}$ mice as described in Materials and methods. (a) Dot-plot illustrating the chimerism in these animals 70 days after transfer using the Ly5.1 and Ly5.2 markers. The data shown are from a $J^{-/-}$ mouse. (b) The ratio between $J^{-/-}$ mice (Ly5.2) and B6.SJL (Ly5.1) cells in the respective compartment is plotted for each animal analysed. The $CD11c^-$ cells represent cells within the live lymphocyte gate. Statistical analysis was performed using Student's *t*-test; $^{***}P < 0{\cdot}001$, $^{**}P < 0{\cdot}01$ and $^{*}P < 0{\cdot}05$.

analysis because the various phenotypes of $J^{-/-}$ mice have shown a gene dosage effect where the heterozygous animal had a partial phenotype.[8,23,24] Figure 3(a) shows representative dot-plots and the gates used for analysis using spleen cells from all three mouse strains, while Fig. 3(b) summarizes the analysis in bar graphs. The percentage of $CD11c^+$ cells was slightly, but not significantly, increased in $J^{-/-}$ mice (left panel). When the $CD11c^+$ cell population was further subdivided a statistically significant change in two populations could be observed. The percentage of $CD4^+$ $CD8\alpha^-$ $CD11c^+$ cells were significantly increased in $J^{-/-}$ mice while the $CD4^-$ $CD8\alpha^+$ population was significantly decreased (middle panel). Interestingly, when spleens from $J^{+/-}$ mice were analysed, an intermediate phenotype was observed with regard to spleen cells from C57BL/6 and $J^{-/-}$ mice as previously observed for other immunological parameters.[23,24]

The pDC compartment is a distinct DC compartment that is $CD11c^{dull}$ but can vary in CD8α expression. We therefore also analysed the impact of J deficiency on $CD11c^+$ cells expressing the pDC marker mPDCA-1. As shown in Fig. 3(b) (right panel), the percentage of this cell population was also significantly reduced compared to C57BL/6 controls, and also in this analysis spleen cells from $J^{+/-}$ mice had an intermediary phenotype. We concluded from these experiments that the absence of J-chain appears to affect the development of a subfration of $CD11c^+$ cells.

To further define the changes in the DC population of $J^{-/-}$ mice we constructed bone marrow chimeras using 50% of the bone marrow cells from B6.SJL (Ly 5.1^+) and 50% from $J^{-/-}$ mice ($Ly5.2^+$). The reconstituted wild-type hosts were analysed after 10 weeks with regard to Ly5 chimerism, as well as the phenotype of $CD11c^+$ and $CD11c^-$ cells. As shown in Fig. 4(a) and (b), while the number of $CD11c^-$ cells within the lymphocyte gate in these chimeras had a 1/1 distribution between cells derived from B6.SJL or $J^{-/-}$ mice, the $CD11c^+$ and mPDCA-1^+ fractions were dominated by cells derived from B6.SJL donors. The most pronounced difference was observed in the mPDCA-1^+ fraction, where the ratio between cells derived from B6.SJL and $J^{-/-}$ bone marrow was 2 to 1. Thus, the reduction of mPDCA-1^+ cells in $J^{-/-}$ mice appears to be intrinsic to the haematopoietic compartment.

$J^{-/-}$ mice have a reduced level of IDO expression

Having noticed that the numbers of $CD8\alpha^+$ and mPDCA-1^+ cells were reduced in $J^{-/-}$ mice, we wanted to investigate whether these animals had a normal expression of the immunoregulatory enzyme IDO that is known to be expressed in these DC subsets.[16] To this end, we purified $CD11c^+$ and $CD11c^-$ cell populations from C57BL/6 and $J^{-/-}$ spleens, prepared RNA and analysed the expression level of IDO mRNA using qRT-PCR. Figure 5(a) shows that the level of IDO RNA in the $CD11c^+$ fraction from $J^{-/-}$ mice was roughly 50% of that seen in C57BL/6 mice, in essence correlating with the reduction of mPDCA-1^+ and $CD4^-/CD8\alpha^+$ DC shown in Fig. 3.

The enzymatic activity of IDO is to break down the essential amino acid tryptophan to kynurenine.[20] An estimate of the overall IDO activity in an animal is therefore reflected in the levels of tryptophan and kynurenine in serum. We therefore compared the levels in C57BL/6 and $J^{-/-}$ mice using HPLC. In Fig. 5(b) these data are shown as the kynurenine/tryptophan ratio. There was a statistically significant difference ($P = 0{\cdot}032$) between the

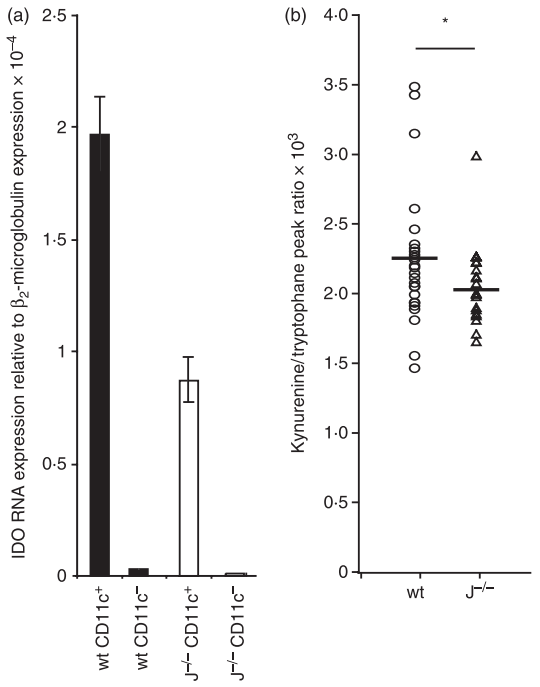

Figure 5. Decreased expression of IDO in $J^{-/-}$ mice. (a) Analysis of RNA expression of IDO using RT-PCR of CD11c fractionated cells (80–90% purity) from the spleens of C57BL/6 and $J^{-/-}$ mice ($n = 6$) relative to β2-microglobulin expression. The error bar represents the standard deviation. (b) Ratio of serum kynurenine over tryptophan from age- and sex-matched wt and $J^{-/-}$ mice are shown. Each symbol represents one mouse and the horizontal bar represents the mean. Statistical analysis was performed using Student's *t*-test; $^{***}P < 0{\cdot}001$, $^{**}P < 0{\cdot}01$ and $^{*}P < 0{\cdot}05$.

kynurenine/tryptophan ratio seen in serum from C57BL/6 mice compared with $J^{-/-}$ mice, indicating a lower overall IDO enzymatic activity in $J^{-/-}$ mice.

We also investigated lymph node sections from C57BL/6 and $J^{-/-}$ mice for IDO and J-chain expression using immune histology. As shown in Fig. 6(a), a subpopulation of $CD11c^+$ cells could be seen that coexpressed J-chain or IDO. Moreover, IDO expression and J-chain expression overlapped to a significant extent, indicating that within the $CD11c^+$ population J-chain was mostly expressed in an IDO^+ subpopulation. When lymph nodes from $J^{-/-}$ mice were stained using the same reagents (Fig. 6b), only background staining was observed with the anti-J-chain antibody. Also, we noted that the number of IDO^+ cells was less in lymph nodes from $J^{-/-}$ mice. Upon quantifying the number of IDO^+ cells per lymph node cross-section in C57BL/6 and $J^{-/-}$ mice, this reduction was substantial (Fig. 6c). To further validate this finding we prepared protein extracts from $CD11c^-$ and $CD11c^+$ populations from C57BL/6 and $J^{-/-}$ mice and analysed these by Western blotting. As shown in Fig. 6(d), while the extracts from both mouse strains contained similar levels of actin, the level of IDO was lower in the extracts from $J^{-/-}$ mice, especially in the $CD11c^+$ cells.

$J^{-/-}$ mice have a deficiency in tolerance induction

$CD8\alpha^+$ DC, pDC and IDO activity have all been associated with regulation of tolerance induction.[14–16] We therefore proceeded to investigate whether the changes

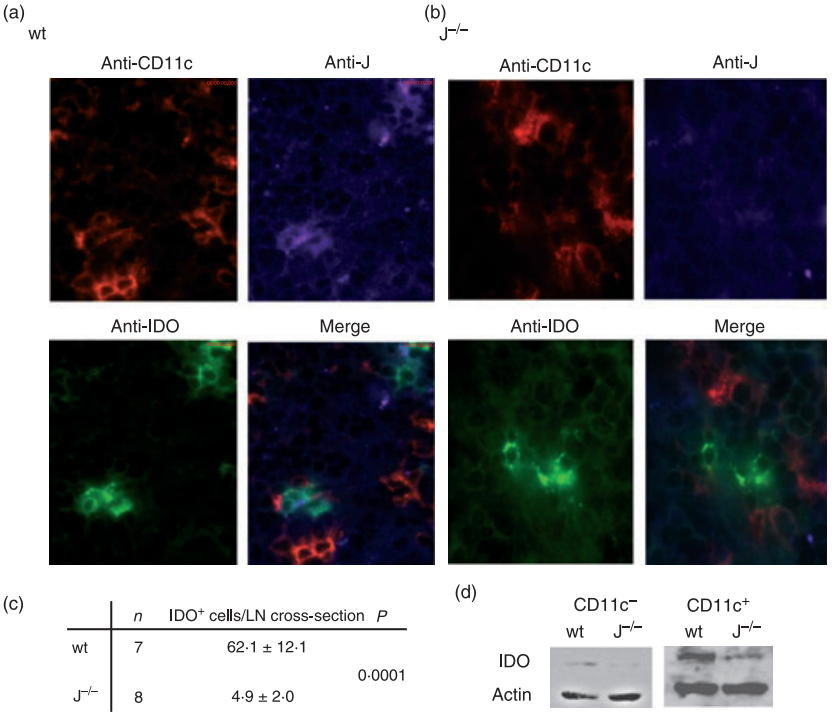

Figure 6. Some $CD11c^+$ cells coexpress J-chain and IDO. (a) Immunfluorescence analysis of CD11c (red), IDO (green) and J-chain (purple) expression in lymph nodes from C57BL/6 and (b) $J^{-/-}$ mice. (c) Summary of IDO^+ cells counted in lymph node cross-sections from C57BL/6 and $J^{-/-}$ mice. Cross-sections were obtained from four different mice. Statistical analysis was performed using Student's *t*-test. (d) Western blot analysis of IDO and actin levels in protein extracts from $CD11c^+$ and $CD11c^-$ cells from spleens of C57BL/6 and $J^{-/-}$ mice.

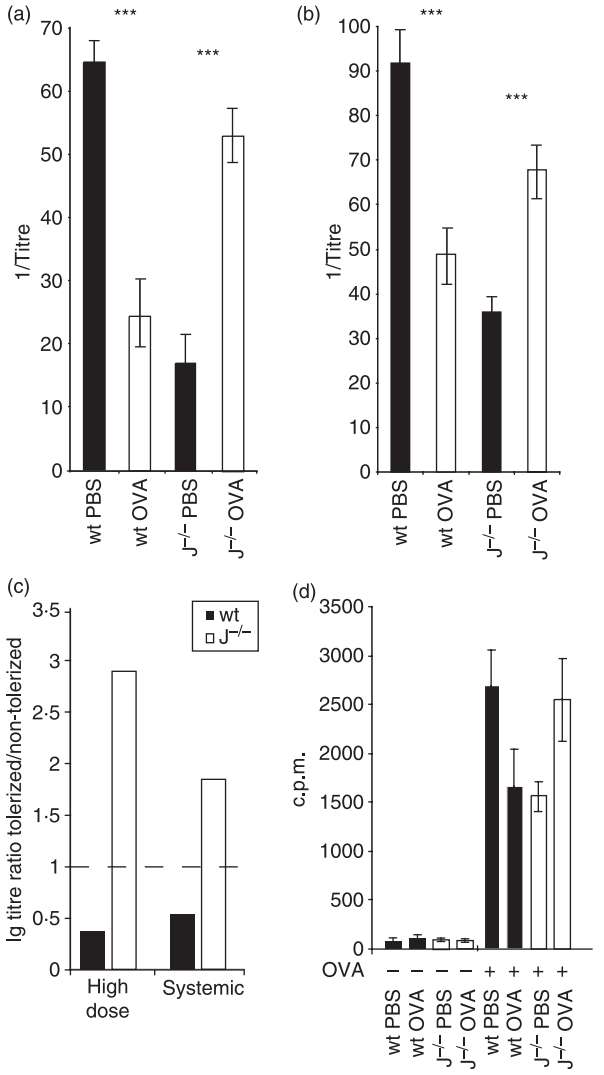

Figure 7. Lack of tolerance in $J^{-/-}$ mice as detected by the serum IgG anti-OVA response. (a) C57BL/6 and $J^{-/-}$ mice were orally tolerized with OVA, or vehicle as a negative control. All mice were subsequently immunized with alum-precipitated OVA intraperitoneally 7 days after tolerization. Mice were killed 14 days after immunization and the serum was analysed for OVA-specific IgG as described in Materials and methods. (b) Systemic tolerance was induced by intravenous injection of OVA and challenged as described above. A minimum of six sex- and age-matched mice were used in each group. The error bar represents the standard deviation. Statistical analysis was performed using Student's *t*-test; $***P < 0{\cdot}001$, $**P < 0{\cdot}01$ and $*P < 0{\cdot}05$. (c) The data from (a) and (b) summarized as the ratio in IgG titre between tolerized and non-tolerized animals. (d) Estimation of OVA-specifc splenic T-cell proliferation in tolerized and non-tolerized C57BL/6 and $J^{-/-}$ mice as described in Materials and methods.

in the DC compartment in $J^{-/-}$ mice had a functional correlate. We compared the effect of oral and systemic tolerization with OVA in $J^{-/-}$ and C57BL/6 mice. Briefly, mice were fed OVA using a high dose (50 mg) of OVA and challenged 14 days later by an intraperitoneal (i.p.) injection of 100 μg OVA in alum. The level of OVA-specific antibody in serum was determined after an additional 2 weeks. As can be seen in Fig. 7(a), the anti-OVA serum response after challenging tolerized wild-type animals was significantly reduced, while in $J^{-/-}$ mice no tolerization was observed. Rather, the immune response after challenge with OVA was increased in $J^{-/-}$ animals tolerized with OVA compared to the non-tolerized control group. Given the role for J-chain in mucosal transport we wanted to confirm the observed phenotype using systemic tolerization and therefore tolerized wild-type and $J^{-/-}$ mice by intravenous injection of OVA and challenged as above. As shown in Fig. 7(b), only C57BL/6 mice were tolerized. Figure 7(c) summarizes the data from the experiments shown in Fig. 7(a) and (b) as the ratio between tolerized and non-tolerized animals, showing that $J^{-/-}$ mice had an aberrant response to tolerogenic challenge. Lastly we wanted to confirm that the effect on serum immunoglobulin levels also could be detected in the T-cell compartment. Hence, we repeated the experiments described above but isolated total T cells from the spleens of treated animals and investigated their proliferatory capacity. As shown in Fig. 7(d), while T-cell proliferation in C57BL/6 mice was reduced in OVA-treated animals compared to controls, T cells from $J^{-/-}$ mice rather showed an increased proliferatory capacity after OVA treatment. Thus, the aberrant response of $J^{-/-}$ mice can also be observed at the level of T-cell proliferation.

Discussion

The current investigation was stimulated by two observations. First, that human DC were shown to express J-chain RNA[10] and, second, that $J^{-/-}$ mice have a deficiency in the formation of B-cell memory that could be adoptively transferred by T cells from $J^{-/-}$ mice.[9] In the latter study we did not observe any differences between the T-cell compartments of C57BL/6 and $J^{-/-}$ mice (E. Källberg and T. Leanderson, unpublished observations).

We have confirmed that DC, defined as $CD11c^+$ cells, in the mouse express J-chain RNA. We then extended this observation and could show that in lymph nodes from normal C57BL/6 mice there is a subpopulation of $CD11c^+$ cells that express also the J-chain protein. The $CD11c^+/J^+$ cells were mostly located outside, but in close proximity to, the B-cell follicles. The function of $CD11c^+/J^+$ cells remains to be defined in detail. However, we show here that a majority of these cells also express IDO, hinting at the possibility that they are involved in immune regulation. That the J-chain protein has a function in these cells is supported by the observation that a reduced number of IDO^+ cells was observed in lymph nodes from $J^{-/-}$ mice. Furthermore, both $CD11c^+$ $CD4^-$ $CD8\alpha^+$ and

$CD11c^+$ $mPDCA\text{-}1^+$ cells were reduced in spleens from $J^{-/-}$ mice compared to C57BL/6 controls, which are the two major IDO-producing cell populations. Thus, J-chain expression in $CD11c^+$ cells appears to occur primarily in the IDO-producing subfraction.

Another observation that is intriguing is the fact that the J-chain locus appears to show a gene dosage phenotype which here was evident in the analysis of DC subsets. We have previously observed this phenomenon in all assays where a phenotype has been observed in $J^{-/-}$ mice. This includes systemic and oral immune responses, as well as serum IgM and IgA steady-state levels in independently generated animals.[8,23,24] We have investigated the possibility that the J-chain locus could be allelically excluded but found this not to be the case (L. Erlandsson and T. Leanderson, unpublished observation). We can at this stage only speculate that J-chain expression needs to reach a critical level to become biologically significant in various cell populations. Clearly this is a finding that merits further study in systems where J-chain expression can be regulated in a quantitative fashion.

We then characterized the $J^{-/-}$ mice further with regard to IDO expression and function. One should here introduce a note of caution. Although our $J^{-/-}$ mice have been backcrossed for 10 generations against C57BL/6 it is impossible to be completely certain that the observed phenotype is the result of the J-chain. A region of genetic material surrounding the J-chain gene is most probably still derived from the I129 strain and could be the cause of the phenotype observed. However, the fact that we have seen the same change in CD11c FACS profiles in three separate backcrosses to C57BL/6, that J-chain mRNA and protein could be detected in a subpopulation of $CD11c^+$ cells from C57BL/6 and $RAG^{-/-}$ mice, and the data from human DC,[10] make us currently work under the assumption that the J-chain itself is the functional moiety behind the observations observed.

It has already been discussed above that a reduced number of IDO^+ cells was observed in lymph node sections from $J^{-/-}$ mice. Furthermore, a reduced level of IDO RNA was also seen in the spleens from $J^{-/-}$ mice compared to C57BL/6. When we estimated the overall IDO activity in $J^{-/-}$ mice by measuring the ratio between tryptophan and kynurenine in serum from healthy animals, a statistically significant reduction was seen in $J^{-/-}$ compared to C57BL/6 mice.

IDO-expressing DC have been shown to be involved in the regulation of immune tolerance.[16,25] We therefore lastly asked the question whether $J^{-/-}$ mice had a functional defect at the level of tolerance induction. We could show that $J^{-/-}$ mice were less prone to be tolerized either orally or systemically. The overall immunological impact of this defect is unclear. We have not observed an increased level of autoimmune disease in these animals, but clearly further studies are needed concerning the effect of the lack of J-chain on autoimmunity and immune function.

In conclusion, we show that a subset of $CD11c^+$ DC in the mouse express J-chain. The exact function of J-chain in this cell population, and at what developmental stage J-chain expression is critical, remain unknown. Future studies should be focused on the clarification of these issues at the biological and biochemical levels.

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References

1 Johansen FE, Pekna M, Norderhaug IN, Haneberg B, Hietala MA, Krajci P, Betsholtz C, Brandtzaeg P. Absence of epithelial immunoglobulin A transport, with increased mucosal leakiness, in polymeric immunoglobulin receptor/secretory component-deficient mice. *J Exp Med* 1999; **190**:915–22.

2 Rojas R, Apodaca G. Immunoglobulin transport across polarized epithelial cells. *Nat Rev Mol Cell Biol* 2002; **3**:944–55.

3 Johansen FE, Braathen R, Brandtzaeg P. The J chain is essential for polymeric Ig receptor-mediated epithelial transport of IgA. *J Immunol* 2001; **167**:5185–92.

4 Brandtzaeg P, Prydz H. Direct evidence for an integrated function of J chain and secretory component in epithelial transport of immunoglobulins. *Nature* 1984; **311**:71–3.

5 Mather EL, Alt FW, Bothwell AL, Baltimore D, Koshland ME. Expression of J chain RNA in cell lines representing different stages of B lymphocyte differentiation. *Cell* 1981; **23**:369–78.

6 Grubb AO. Quantitation of J chain in human biological fluids by a simple immunochemical procedure. *Acta Med Scand* 1978; **204**:453–65.

7 Brandtzaeg P. Immunohistochemical characterization of intracellular J-chain and binding site for secretory component (SC) in human immunoglobulin (Ig)-producing cells. *Mol Immunol* 1983; **20**:941–66.

8 Erlandsson L, Akerblad P, Vingsbo-Lundberg C, Kallberg E, Lycke N, Leanderson T. Joining chain-expressing and -nonexpressing B cell populations in the mouse. *J Exp Med* 2001; **194**:557–70.

9 Kallberg E, Leanderson T. Joining-chain (J-chain) negative mice are B cell memory deficient. *Eur J Immunol* 2006; **36**:1398–1403.

10 Rissoan MC, Duhen T, Bridon JM, Bendriss-Vermare N, Peronne C, de Saint Vis B, Briere F, Bates EE. Subtractive hybridization reveals the expression of immunoglobulin-like transcript 7, Eph-B1, granzyme B, and 3 novel transcripts in human plasmacytoid dendritic cells. *Blood* 2002; **100**:3295–303.

11 Moser M. Dendritic cells in immunity and tolerance – do they display opposite functions? *Immunity* 2003; **19**:5–8.

12 Hackstein H, Thomson AW. Dendritic cells: emerging pharmacological targets of immunosuppressive drugs. *Nat Rev Immunol* 2004; **4**:24–34.

13 Shortman K, Naik SH. Steady-state and inflammatory dendritic-cell development. *Nat Rev Immunol* 2007; **7**:19–30.
14 Hawiger D, Inaba K, Dorsett Y *et al.* Dendritic cells induce peripheral T cell unresponsiveness under steady state conditions *in vivo*. *J Exp Med* 2001; **194**:769–79.
15 Munn DH, Zhou M, Attwood JT, Bondarev I, Conway SJ, Marshall B, Brown C, Mellor AL. Prevention of allogeneic fetal rejection by tryptophan catabolism. *Science* 1998; **281**:1191–3.
16 Mellor AL, Munn DH. IDO expression by dendritic cells: tolerance and tryptophan catabolism. *Nat Rev Immunol* 2004; **4**:762–74.
17 Mellor AL, Baban B, Chandler P *et al.* Cutting edge: induced indoleamine 2,3 dioxygenase expression in dendritic cell subsets suppresses T cell clonal expansion. *J Immunol* 2003; **171**:1652–5.
18 Soumelis V, Liu YJ. From plasmacytoid to dendritic cell: morphological and functional switches during plasmacytoid pre-dendritic cell differentiation. *Eur J Immunol* 2006; **36**:2286–92.
19 Kallberg E, Leandersson T. Analysis of antigen-specific and naturally occurring IgM and IgA steady-state levels in J-chain negative C57BL/6 mice. *Scand J Immunol* 2006; **63**:430–4.
20 Laich A, Neurauter G, Widner B, Fuchs D. More rapid method for simultaneous measurement of tryptophan and kynurenine by HPLC. *Clin Chem* 2002; **48**:579–81.
21 Munn DH, Sharma MD, Hou D *et al.* Expression of indoleamine 2,3-dioxygenase by plasmacytoid dendritic cells in tumor-draining lymph nodes. *J Clin Invest* 2004; **114**:280–90.
22 Pelayo R, Hirose J, Huang J, Garrett KP, Delogu A, Busslinger M, Kincade PW. Derivation of 2 categories of plasmacytoid dendritic cells in murine bone marrow. *Blood* 2005; **105**:4407–15.
23 Erlandsson L, Andersson K, Sigvardsson M, Lycke N, Leanderson T. Mice with an inactivated joining chain locus have perturbed IgM secretion. *Eur J Immunol* 1998; **28**:2355–65.
24 Lycke N, Erlandsson L, Ekman L, Schon K, Leanderson T. Lack of J chain inhibits the transport of gut IgA and abrogates the development of intestinal antitoxic protection. *J Immunol* 1999; **163**:913–9.
25 Kapturczak MH, Wasserfall C, Brusko T, Campbell-Thompson M, Ellis TM, Atkinson MA, Agarwal A. Heme oxygenase-1 modulates early inflammatory responses: evidence from the heme oxygenase-1-deficient mouse. *Am J Pathol* 2004; **165**:1045–53.