Immunobiology 220 (2015) 769–774

Contents lists available at ScienceDirect

Immunobiology

journal homepage: www.elsevier.com/locate/imbio

Short Communication

Retinaldehyde dehydrogenase activity is triggered during allograft rejection and it drives Th1/Th17 cytokine production

Rodrigo A. Morales[a], Mauricio Campos-Mora[a], Tania Gajardo[a], Francisco Pérez[a], Javier Campos[a], Juan C. Aguillón[b], Karina Pino-Lagos[a,*]

[a] Programa Disciplinario de Inmunología, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Santiago, Chile
[b] Immune Regulation and Tolerance Research Group, Programa Disciplinario de Inmunología, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Santiago, Chile[1]

ARTICLE INFO

Article history:
Received 25 November 2014
Received in revised form
22 December 2014
Accepted 23 December 2014
Available online 3 January 2015

Keywords:
Retinoic acid
Rejection
Th1/Th17
Transplantation

ABSTRACT

Retinoic acid (RA), a vitamin A metabolite, has been attributed to relevant functions in adaptive immunity. On T cells, the disruption on RA signaling alters both CD4+ and CD8+ T cells effector function. In this study, we evaluated the contribution of RA synthesis during the immune response using an *in vivo* skin transplantation model. Our data indicates that the frequency and number of cells containing an active retinaldehyde dehydrogenase (RALDH), a key enzyme for RA synthesis, is increased during skin transplant rejection. In addition, we found that the expression of the mRNA coding for the isoform RALDH2 is up-regulated on graft rejecting draining lymph nodes (dLNs) cells. Lastly, we observed that IFN-γ and IL-17 production by *ex vivo* re-stimulated dLNs cells is greatly increased during rejection, which it turns depends on RA synthesis, as shown in experiments using a specific RALDH inhibitor. Altogether, our data demonstrate that RA synthesis is incremented during the immune response against an allograft, and also indicates that the synthesis of RA is required for cytokine production by dLNs resident T cells.

Introduction

Retinoic acid (RA), one of the vitamin A metabolites, plays a vast array of functions in the immune system. RA corresponds to the metabolically active form of vitamin A, and it is catabolized *via* enzymatic reactions, in which the retinaldehyde dehydrogenase (RALDH) family exerts the last step in an irreversible fashion (Duester, 2000). After its synthesis in the cytoplasm, RA is translocated to the nucleus where it binds to the RA- and RX-receptors complex (RARs/RXRs), to next recognize target DNA sequences to regulate gene expression (Bastien and Rochette-Egly, 2004).

Although, many decades ago the relevance of vitamins in health was almost intuitive, it was not until the early 80s, taking advantage of the first experimental approaches using vitamin A deficient animals, that some studies indicated that vitamin A and RA are important for T and B cell mediated immunity (Sirisinha et al., 1980; Smith et al., 1987). The development of molecular technologies such as molecules with agonists/antagonists function, and genetically manipulated animals, permitted to re-discover the contribution of RA in immune cells and in pathological *in vivo* animal models (Hall et al., 2011b). Up to date, it is known that bone marrow-derived and splenic CD11c+ dendritic cells (DCs) up-regulate RALDH activity upon GM-CSF and IL-4 treatment (Yokota et al., 2009), while DCs from intestinal tract and skin dLNs express RALDH constitutively (Coombes et al., 2007; Guilliams et al., 2010; Iwata et al., 2004; Sun et al., 2007). Meanwhile, not only DCs have been attributed RALDH activity or RA synthesis, but also stromal cells and basophils (Hammerschmidt et al., 2008; Molenaar et al., 2009; Spiegl et al., 2008). Conversely, the role of RA signaling on T cells has been recently described in at least 4 *in vivo* models, such as transplantation, tumor, infection and graft-versus-host disease (GvHD) using either RARα KO mice

Abbreviations: APC, antigen presenting cell; DCs, dendritic cells; DEAB, diethylaminobenzaldehyde; dLNs, draining-lymph nodes; ELISA, enzyme linked immunosorbent assay; IFN, interferon; GM-CSF, granulocyte-macrophage colony stimulating factor; GvHD, graft-versus-host disease; IL, interleukin; ILCs, innate lymphoid cells; PCR, polymerase chain reaction; qRT-PCR, quantitative reverse transcriptase-PCR; RA, retinoic acid; RALDH, retinaldehyde dehydrogenase; RAR, retinoic acid receptor; RXR, retinoic-X-receptor; Th, T helper.

* Corresponding author at: Programa Disciplinario de Inmunología, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Av. Independencia 1027, 3er Piso, Pabellón I, Santiago 8380453, Chile. Tel.: +56 2 2978 9503; fax: +56 2 2978 6979.
E-mail address: karina.p.lagos@gmail.com (K. Pino-Lagos).
[1] www.irtgroup.cl.

http://dx.doi.org/10.1016/j.imbio.2014.12.018
0171-2985/

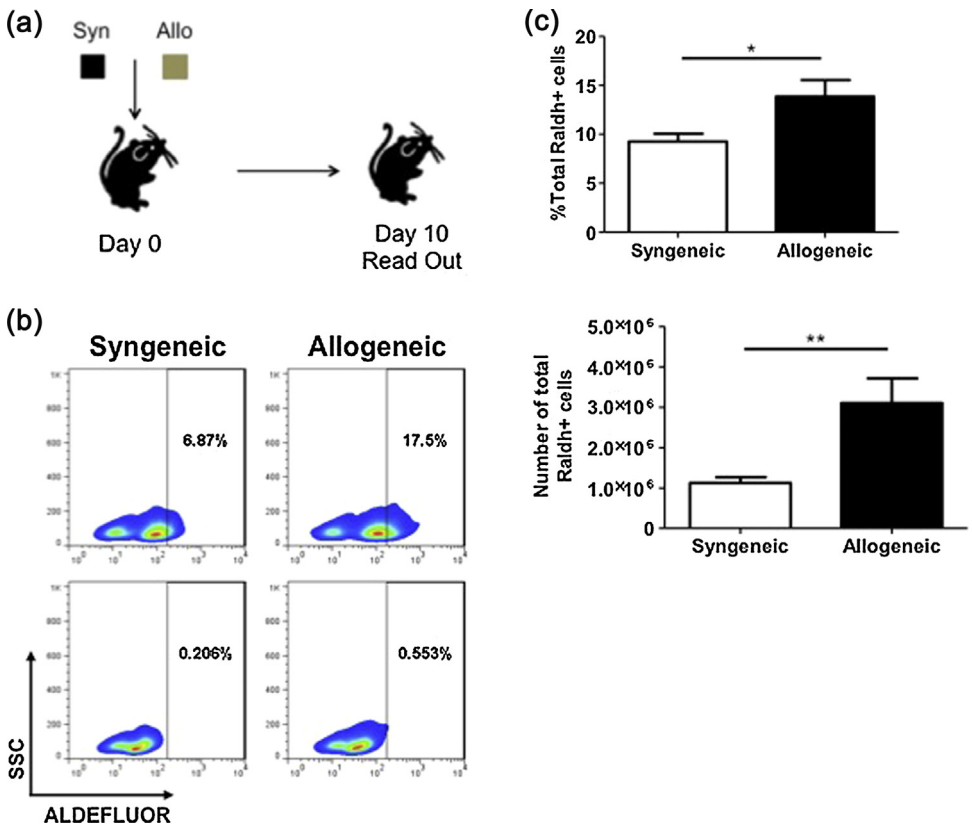

Fig. 1. Retinoic acid synthesis augments during allograft rejection. (a) The picture depicts the skin transplant model, where C57Bl/6 recipient mice receive either C57Bl/6 syngeneic or F1 (C57Bl/6 × Balb/c) allogeneic skin grafts at day 0. Mice and transplants are monitored 2 times per week until day 10 where the animals are euthanized for tissue and organ collection. (b) Characteristic density plots showing the frequencies of RALDH+ cells in syngeneic and allogeneic groups (top). At the bottom, it is shown the negative controls obtained by incubating the cells with the Raldh inhibitor, DEAB. (c) Graphs displaying the frequencies (top) and numbers (bottom) of RALDH+ cells. Bars correspond to the standard error of the mean (SEM), and the statistical significance was assessed by paired *t* test. $^{*}p < 0.05$; $^{**}p < 0.01$. $n = 4$ independent experiments, with 2–3 mice per group in each experiment.

or by enhancing the expression of a dominant negative form of RARα (dnRARα) on T cells (Chen et al., 2013; Guo et al., 2012; Hall et al., 2011a; Pino-Lagos et al., 2011). Based on these studies, one can conclude that: (i) RA signaling is triggered during the initiation and progression of inflammation, (ii) deficiency of RA ameliorates inflammatory responses by impairing effector T cell function, (iii) CD4+ T cells need to sense RA to differentiate into Th1 and Th17 cells, (iv) CD8+ T cells require RA for expansion, survival and anti-tumor activity. Although the evidence mentioned above highlights that the disruption of RA signaling impairs T cells function, it is not well comprehend whether RA synthesis by RALDH-expressing cells is relevant during certain inflammatory conditions.

In this short communication we used an *in vivo* model of skin transplantation to study the potential changes on RA synthesis during the immune response against the allograft. Our data shows that RALDH expression and activity increment during allograft rejection. Additionally, we showed that myeloid cells express a metabolically active RALDH, but do not correspond to total RALDH+ cells. According with these results, and previously published data, allogeneic Th1/Th17 cells activities, given by the secretion of IFN-γ and IL-17 from *ex vivo*-stimulated dLNs cells, respectively, require RA since the production of these cytokines is importantly reduced when the cells are cultured in the presence of a RALDH inhibitor. Taking all together, our results suggest that RA synthesis occurs during allograft rejection and, most likely, T cells sense this RA, which is in turn required for T cell mediated immunity leading to transplant rejection.

Materials and methods

Mice

Six to 8 weeks old C57Bl/6 wild type mice were used in this study as graft recipients and syngeneic skin graft donors. F1 ($H2^{dxb}$) mice, which correspond to skin allograft donors, were obtained by crossing BALB/c mice ($H2^{d}$) with WT C57Bl/6 mice ($H2^{b}$). Mice were maintained in accordance with the Bioethical Committee guidelines from the Facultad de Medicina, Universidad de Chile.

Skin transplantation

Skin grafting was performed as described previously (Campos-Mora et al., 2014). Briefly, tail skin (~1 cm^2) from C57Bl/6 (syngeneic) or F1 (allogeneic) donors was transplanted onto the dorsal area of C57Bl/6 recipient mice. At day 10 post surgery, draining lymph nodes (dLNs) from graft recipients were obtained for further analysis. The integrity of skin grafts was evaluated 2 times per week, and grafts were considered rejected when 80% of the original graft disappeared or became necrotic, as previously described (Quezada et al., 2005).

RALDH activity analysis

For detection of RALDH activity, cells from dLNs were stained with ALDEFLUOR reagent (StemCell Technologies, Vancouver, Canada) following the manufacturer's instructions, but with one modification included, which corresponded to the addition of the

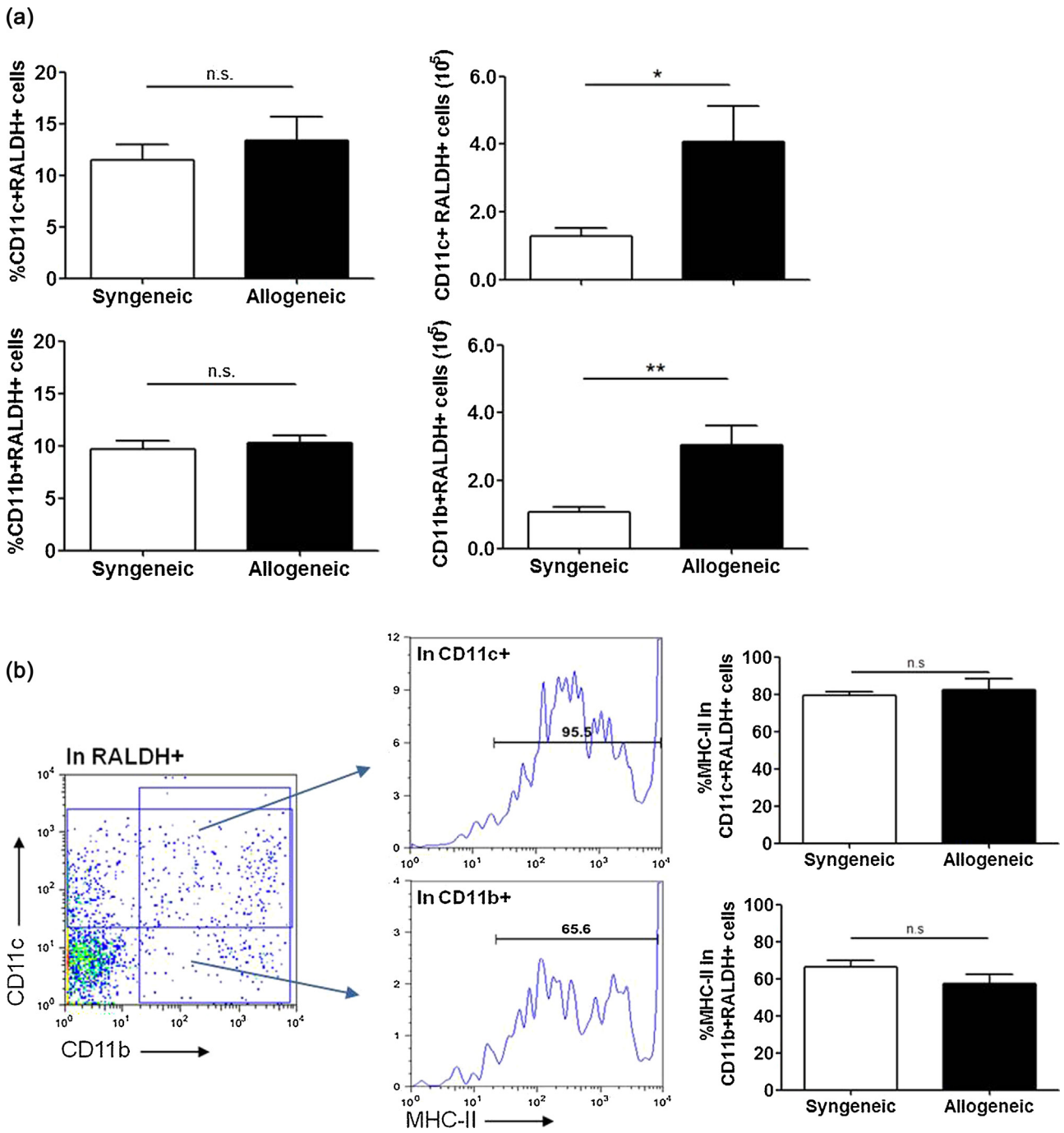

Fig. 2. RALDH+ cells include CD11c+ and CD11b+ cells subsets in dLNs. (a) Expression of CD11c and CD11b in dLNs was analyzed 10 days after transplantation. The percentage (left) and total number (right) of RALDH+CD11c+ (top) and RALDH+CD11b+ (bottom) cells are shown. (b) Representative dot plot showing the staining for CD11c and CD11b in previously gated RALDH+ cells from dLNs. On the right, histograms display MHC-II expression on total CD11c (top) or CD11b (bottom), and the frequencies of these cells are shown in the adjacent graphs (right). Bars correspond to standard error of the mean (SEM), and the statistical significance was assessed by paired *t* test. $^{*}p < 0.05$; $^{**}p < 0.01$. For (a) $n = 4$ independent experiments, with 2–3 mice per group in each experiment. For (b) $n = 1$ experiment, with 4 mice per group.

RALDH inhibitor diethylaminobenzaldehyde (DEAB) to the cells 5 min before ALDEFLUOR staining. Flow cytometry data acquisition was performed with FACSCalibur using CellQuest software (Beckton Dickinson, Mountain View, CA, USA). Data were analyzed using FlowJo software (TreeStar Inc., Ashland, OR, USA).

qRT-PCR

The RNA was extracted from dLNs cells at day 10 post-transplantation using RNeasy kit (Qiagen, Hilden, Germany). cDNA samples were prepared with iScript cDNA synthesis kit (Bio-Rad, Hercules, CA, USA). Expression of the three RALDH isoforms was performed in a Mx3000P qPCR system (Agilent Technologies, Santa Clara, CA, USA) using iQ SYBR Green Supermix (Bio-Rad) as fluorescent detector and specific primers for *raldh1* (5′-ATGGTTTAGCAGCAGCAGTCTTC-3′ and 5′-CCAGACATCTTGAATCCACC-GAA-3′), *raldh2* (5′-GACTTGTA-GCAGCTGTCTTCACT-3′ and 5′-TCACCCATTTCT-CTCCCATTTCC-3′), *raldh3* (5′-GGACAGTCTGGATCAACTGCTAC-3′ and 5′-TCAGGGGTT-CTTCTCCTCGAGT-3′) and *gapdh* (5′-CCAGGTTGTCTCCTGC-GACTT-3′ and 5′-CCTGTTGCTGTAGCCGTATTCA-3′), as described previously (Iwata et al., 2004). For analysis, the expression of RALDH

isoforms was normalized with respect to the housekeeping gene GAPDH.

Recall experiments

dLNs obtained 10 days after surgery were processed and cell suspensions were obtained and concentrated at 1×10^6 cells/mL/well in RPMI-1640 medium (Gibco BRT, Grand Island, NY, USA) supplemented with 10 mM HEPES, 100 IU/mL penicillin/streptomycin, 10% heat-inactivated fetal bovine serum (FBS, Gibco BRT, Grand Island, NY, USA) and 50 μM 2β-mercaptoethanol (Sigma–Aldrich, St. Louis, MO, USA). Cells were seeded in 24-well plates (BD Biosciences, San Jose, CA, USA) in the presence of polyclonal activation (5 μg/mL αCD3 clone 2c11, Biolegend, San Diego, CA, USA) with or without DEAB (15 μM, StemCell Technology, Vancouver, Canada) for 3 days at 37 °C and 5% CO_2.

ELISA test

Supernatants were collected and stored at −80 °C until cytokine quantification by ELISA (sandwich) test using α-IFN-γ and α-IL-17 antibodies (all from eBioscience, San Diego, CA, USA). Recombinant murine cytokines for standard curves were obtained from Peprotech (Rocky Hill, NJ, USA).

Statistics

Data were analyzed using unpaired Student's *t*-tests. In all cases, $p < 0.05$ was considered with statistical significance. For data analysis, GraphPad Prism v5.0 (GraphPad Software, San Diego, CA, USA) was used.

Results

Raldh activity and expression are increased during allograft rejection

RA signaling gets triggered during inflammatory processes (Aoyama et al., 2013; Hall et al., 2011a; Pino-Lagos et al., 2011) For example, in transplantation, it has been shown that CD4+ T cells from rejecting dLNs can sense RA in the nucleus, and this signal is crucial for triggering Th1/Th17 cell responses (Pino-Lagos et al., 2011). However, it is not clear if those dLNs have cells capable to synthesize RA. Using a model of tail skin transplantation, in which C57Bl/6 recipient mice received either syngeneic (C57Bl/6) or allogeneic F1 (C57Bl/6 × Balb/c) skin grafts (Fig. 1a), we assessed the ability of graft dLNs cells to synthesize RA. For this aim, we used ALDEFLUOR system, a very established and accepted technique which permits the identification of cells expressing a metabolically active RALDH, or in other words, allows for the identification and phenotyping of cells synthesizing RA by flow cytometry. At

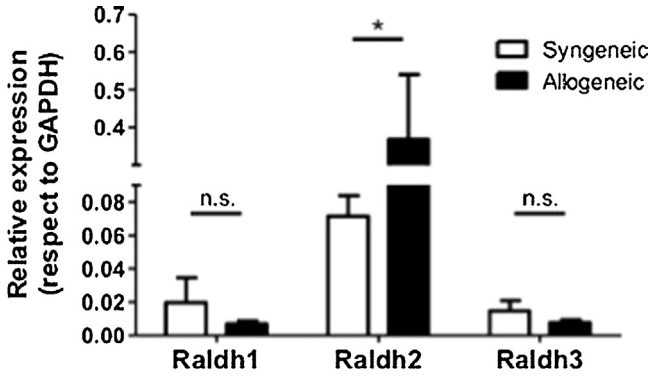

Fig. 3. RALDH2 is the main isoform expressed by rejecting-dLNs cells. mRNA for *Raldh* isoforms was quantified in graft dLNs cells (from syngeneic and allogeneic groups) by qRT-PCR. GAPDH corresponded to the housekeeping gene (internal control). Bars correspond to standard error of the mean (SEM), and the statistical significance was assessed by paired *t* test. $^{**}p < 0.01$. $n = 3$ independent experiments, with 2–3 mice per group in each experiment.

day 10 post-transplantation, we found an increase in the percentage of RALDH+ cells during allograft rejection in comparison to the syngeneic counterpart (13.86% ± 1.68% versus 9.25% ± 0.81% respectively), Fig. 1b and c, top graph). This increment was much significant if we compare cell number (from ∼1×10^6 in syngeneic to ∼3×10^6 in allogeneic condition, Fig. 1c, bottom graph). Given that skin-derived DCs express RALDH in dLNs (Guilliams et al., 2010) we evaluated the phenotype of RALDH+ cells. We found that CD11c+ cells represent a fraction of RALDH+ cells (11.44% ± 1.54% in syngeneic and 13.38% ± 2.30% in allogeneic condition, Fig. 2a, top), and during graft rejection the number of CD11c+ cells increased, but maintaining the frequency compared to syngeneic (Fig. 2a, top right plot). Following the same trend that CD11c+ RALDH+ cells, when we extended the analysis to CD11b+ cells, we noted that 9.69% ± 0.77% and 10.25% ± 0.70% of CD11b+ cells were RALDH+ in syngeneic and allogeneic condition, respectively (Fig. 2a bottom), but the number of CD11b+RALDH+ cells augments during graft rejection, maintaining their frequency (Fig. 2a, bottom right plot), suggesting that dLNs infiltration by CD11c+/CD11b+RALDH+ may account for a higher RALDH activity during transplant rejection. To evaluate the phenotype of these CD11c/CD11b+ RALDH+ cells, we included staining for MHC-II as well, obtaining that ∼90% of the CD11c+RALDH+ co-express MHC-II, Fig. 2b top. Similarly, CD11b+RALDH+ cells also co-express MHC-II but at a lower frequency ∼70%. These data indicates that APCs, such as DCs, macrophages, display RALDH activity during allograft rejection.

Next, we evaluated *Raldh*s mRNA levels in dLNs cells from syngeneic and allogeneic transplanted mice. The qRT-PCR analysis of graft dLNs cells showed that *Raldh2* mRNA level was higher than the *Raldh1* and *Raldh3* isoforms in syngeneic mice (<0.3 for *Raldh* 1 and 3 versus >0.5 for *Raldh* 2, Fig. 3). Complementing the flow cytometry

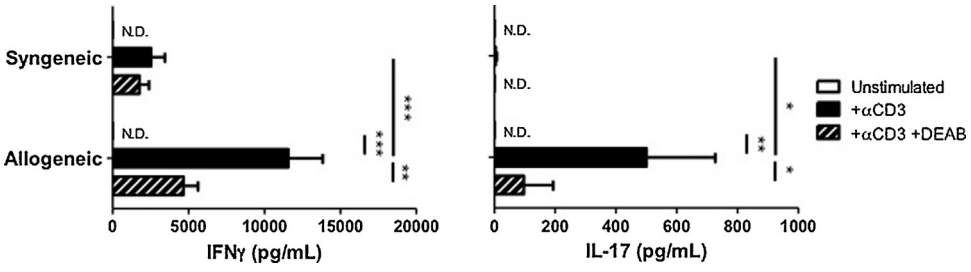

Fig. 4. Rejecting dLNs-resident T cells produce Th1/Th17 cytokines in a RA synthesis-dependent manner. Skin transplants were performed as described earlier. At day 10, graft dLNs were removed to obtain cell suspensions, which were cultured under polyclonal activation (αCD3), in the presence or absence of DEAB (15 μM). Graphs showing the amounts of IFN-γ (left) and IL-17 (right) produced by *ex vivo*-stimulated dLNs cells. Bars correspond to the standard error of the mean (SEM), and the statistical significance was assessed by two-way ANOVA and Bonferroni post-test. $^{*}p < 0.05$; $^{**}p < 0.01$. $n = 2$ independent experiments, with 2–4 mice per group in each experiment.

data, *Raldh* 2 mRNA expression resulted higher during allograft rejection versus the syngeneic condition (~3-fold increase, Fig. 3). These data indicates that allograft-driven inflammation triggers RA synthesis, and *Raldh2* isoform corresponds to the enzyme responsible for RALDH activity in dLNs cells.

RA synthesis triggered during allograft rejection is required to produce Th1/Th17 cytokines

As mentioned above, both CD4+ and CD8+ T cells need to sense RA to exert their effector function. Therefore, to link this finding with the RALDH activity observed in our previous experiments, we decided to evaluate the contribution of RALDH on *ex vivo* stimulated dLNs cells. Thus, we obtained dLNs cells at day 10 post-transplantation, and prepared cell suspensions to culture under polyclonal activation (αCD3) in the presence or absence of the RALDH activity inhibitor DEAB. After 3 days of incubation, the secretion of IFN-γ and IL-17 was measured in the supernatants using ELISA. Importantly, we did not see a negative impact on cell viability due to the inclusion of the DEAB inhibitor in the cell cultures (data not shown). As shown in Fig. 4, we observed that activated dLNs cells from allogeneic mice secreted high levels of Th1/Th17 related cytokines (~11,000 pg/mL for IFN-γ and ~500 pg/mL for IL-17), compared to the syngeneic (≤5000 pg/mL for IFN-γ and ≤20 pg/mL for IL-17) and non-activated controls (for both cytokines non-detectable). Furthermore, the blockade of RALDH activity with DEAB inhibited the secretion of IFN-γ (from ~11,000 pg/mL to ~5000 pg/mL [+DEAB]) and IL-17 (from ~500 pg/mL to ~100 pg/mL [+DEAB]) suggesting that RA synthesis is necessary for the development of Th1/Th17 cell responses during graft rejection.

Discussion

During the last ten years, the literature regarding the role of vitamin A, specifically RA, has been explosive, mainly due to the development of new technologies to evaluate the effect of RA at the cellular level, without mentioning the use of animal models to mimic human disease. In this regard, the studies on the contribution of RA in adaptive immunity indicate that RA affects lymphocyte biology by targeting several functions of B and T cells. For example, RA imprints the gut homing markers CCR9 and α4β7 on CD8+, CD4+ and B cells directing their migratory properties to the gut (Iwata et al., 2004; Mora et al., 2006; Svensson et al., 2008); RA helps B cells in their differentiation toward plasma cells and improves antibody production (Mora et al., 2006); RA is required for CD8+ T cell survival and anti-tumor effector activity (Guo et al., 2012, 2014); and the interruption of RA signaling on CD4+ T cells allows for allograft tolerance, partially by restraining Th1 and Th17 differentiation (Pino-Lagos et al., 2011). Conversely, the source of RA has been mainly granted to gut mucosa DCs, although other cell types, such as stromal cells and basophils, have been identified as RA producers as well (Molenaar et al., 2009; Spiegl et al., 2008). If we summarize previous studies, where the recognition of RA is disrupted, we can state that T cells need RA for their function, but we still do not know if RA synthesis takes place under physiological conditions or if RA synthesis is important for immunological processes. In this short communication, we tested for RA synthesis, or RALDH activity in the dLNs of transplanted animals. Our data indicates that under inflammation triggered by allograft rejection, there is an up-regulation on the frequency and number of RALDH+ cells, suggesting that infiltrating and/or proliferating cells in the lymph nodes (including RALDH+ cells or those turning on RALDH activity once in the dLNs due to the microenvironment) cooperate as a source of RA. The above is supported by Pino-Lagos et al. report where it is demonstrated that either immune vaccination or allograft rejection triggers RA signaling on CD4+ T cells, or in other words, the inflammatory environment turns RA signaling on, permitting that local cells can access to and sense RA. Moreover, and in line with previous results (Guo et al., 2012; Aoyama et al., 2013), we found that RALDH+ cells correspond to CD45+ leukocytes (Supplementary Fig. 1), mainly APCs from the CD11c+MHC-II+ and CD11b+MHC-II+ subsets, but they do not represent the entire population of RALDH+ cells, suggesting that other immune cells, such as innate lymphoid cells (ILCs), which express MHC-II and are RA sensitive (McKenzie et al., 2014) may display RALDH activity.

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.imbio.2014.12.018.

As stated earlier, RALDH enzymes can be distinguished among three isoforms, RALDH1-3 (Duester, 2000). In our work we found that RALDH2 can be the responsible for RA synthesis in dLNs. It has been shown that RALDH2 is essential for embryonic development (Duester, 2001), and in immune cells, RALDH2 has been attributed to DCs from mesenteric lymph nodes and also stromal cells (Iwata et al., 2004; Molenaar et al., 2009). These data indicate that in dLNs there are not only RA-sensing cells, such as T cells, but also RA synthesis takes place in the graft dLNs during the allo-response and appears to be essential for T cell function. If we combine these two pieces, the picture proposes that cells in dLNs produce RA and T cells can sense it to differentiate into effector T cells. Despite of impairing RA signaling on T cells, can we get Th1/Th17 differentiation if we block RA synthesis? Our data suggest that indeed, by using a RALDH inhibitor, we can alter IFN-γ and IL-17 production implying that T cells may detect RA produced by other cells since T cells do not become RALDH+ under steady state or activation conditions (data not shown). Alternatively, by blocking RALDH activity we could be affecting DCs biology as well because RA signaling on DCs is associated with their maturation and antigen presentation, enhancing T cell responses (Geissmann et al., 2003). Further experiments are required to explain the molecular mechanisms involved in effector T cell polarization by RA synthesis.

Taking together, our results confirm that RA is involved in inflammation and immune activation. Furthermore, we described for the first time that RA synthesis takes place when the immune system responds against an allograft, which in turns can affect T cell biology.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

We are very grateful to Dr. Arturo Ferreira for facilitating the performance of some experiments (facility/equipment) and to Mrs. Ruth Mora for technical assistance.

This work was supported by FONDECYT Grant 11121309.

References

Aoyama, K., Saha, A., Tolar, J., Riddle, M.J., Veenstra, R.G., Taylor, P.A., Blomhoff, R., Panoskaltsis-Mortari, A., Klebanoff, C.A., Socie, G., et al., 2013. Inhibiting retinoic acid signaling ameliorates graft-versus-host disease by modifying T-cell differentiation and intestinal migration. Blood 122, 2125–2134.

Bastien, J., Rochette-Egly, C., 2004. Nuclear retinoid receptors and the transcription of retinoid-target genes. Gene 328, 1–16.

Campos-Mora, M., Morales, R.A., Perez, F., Gajardo, T., Campos, J., Catalan, D., Aguillon, J.C., Pino-Lagos, K., 2014. Neuropilin-1 regulatory T cells promote skin allograft survival and modulate effector CD4 T cells phenotypic signature. Immunol. Cell Biol., http://dx.doi.org/10.1038/icb.2014.77 [Epub ahead of print].

Chen, X., Dodge, J., Komorowski, R., Drobyski, W.R., 2013. A critical role for the retinoic acid signaling pathway in the pathophysiology of gastrointestinal graft-versus-host disease. Blood 121, 3970–3980.

Coombes, J.L., Siddiqui, K.R., Arancibia-Carcamo, C.V., Hall, J., Sun, C.M., Belkaid, Y., Powrie, F., 2007. A functionally specialized population of mucosal CD103+ DCs

induces Foxp3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. J. Exp. Med. 204, 1757–1764.
Duester, G., 2000. Families of retinoid dehydrogenases regulating vitamin A function: production of visual pigment and retinoic acid. Eur. J. Biochem. 267, 4315–4324.
Duester, G., 2001. Genetic dissection of retinoid dehydrogenases. Chem. Biol. Interact. (130–132), 469–480.
Geissmann, F., Revy, P., Brousse, N., Lepelletier, Y., Folli, C., Durandy, A., Chambon, P., Dy, M., 2003. Retinoids regulate survival and antigen presentation by immature dendritic cells. J. Exp. Med. 198, 623–634.
Guilliams, M., Crozat, K., Henri, S., Tamoutounour, S., Grenot, P., Devilard, E., de Bovis, B., Alexopoulou, L., Dalod, M., Malissen, B., 2010. Skin-draining lymph nodes contain dermis-derived CD103(−) dendritic cells that constitutively produce retinoic acid and induce Foxp3(+) regulatory T cells. Blood 115, 1958–1968.
Guo, Y., Lee, Y.C., Brown, C., Zhang, W., Usherwood, E., Noelle, R.J., 2014. Dissecting the role of retinoic acid receptor isoforms in the CD8 response to infection. J. Immunol. 192, 3336–3344.
Guo, Y., Pino-Lagos, K., Ahonen, C.A., Bennett, K.A., Wang, J., Napoli, J.L., Blomhoff, R., Sockanathan, S., Chandraratna, R.A., Dmitrovsky, E., et al., 2012. A retinoic acid-rich tumor microenvironment provides clonal survival cues for tumor-specific CD8(+) T cells. Cancer Res. 72, 5230–5239.
Hall, J.A., Cannons, J.L., Grainger, J.R., Dos Santos, L.M., Hand, T.W., Naik, S., Wohlfert, E.A., Chou, D.B., Oldenhove, G., Robinson, M., et al., 2011a. Essential role for retinoic acid in the promotion of CD4(+) T cell effector responses via retinoic acid receptor alpha. Immunity 34, 435–447.
Hall, J.A., Grainger, J.R., Spencer, S.P., Belkaid, Y., 2011b. The role of retinoic acid in tolerance and immunity. Immunity 35, 13–22.
Hammerschmidt, S.I., Ahrendt, M., Bode, U., Wahl, B., Kremmer, E., Forster, R., Pabst, O., 2008. Stromal mesenteric lymph node cells are essential for the generation of gut-homing T cells in vivo. J. Exp. Med. 205, 2483–2490.
Iwata, M., Hirakiyama, A., Eshima, Y., Kagechika, H., Kato, C., Song, S.Y., 2004. Retinoic acid imprints gut-homing specificity on T cells. Immunity 21, 527–538.
McKenzie, A.N.J., Spits, H., Eberl, G., 2014. Innate lymphoid cells in inflammation and immunity. Immunity 41, 366–374.
Molenaar, R., Greuter, M., van der Marel, A.P., Roozendaal, R., Martin, S.F., Edele, F., Huehn, J., Forster, R., O'Toole, T., Jansen, W., et al., 2009. Lymph node stromal cells support dendritic cell-induced gut-homing of T cells. J. Immunol. 183, 6395–6402.
Mora, J.R., Iwata, M., Eksteen, B., Song, S.Y., Junt, T., Senman, B., Otipoby, K.L., Yokota, A., Takeuchi, H., Ricciardi-Castagnoli, P., et al., 2006. Generation of gut-homing IgA-secreting B cells by intestinal dendritic cells. Science 314, 1157–1160.
Pino-Lagos, K., Guo, Y., Brown, C., Alexander, M.P., Elgueta, R., Bennett, K.A., De Vries, V., Nowak, E., Blomhoff, R., Sockanathan, S., et al., 2011. A retinoic acid-dependent checkpoint in the development of CD4+ T cell-mediated immunity. J. Exp. Med. 208, 1767–1775.
Quezada, S.A., Bennett, K., Blazar, B.R., Rudensky, A.Y., Sakaguchi, S., Noelle, R.J., 2005. Analysis of the underlying cellular mechanisms of anti-CD154-induced graft tolerance: the interplay of clonal anergy and immune regulation. J. Immunol. 175, 771–779.
Sirisinha, S., Darip, M.D., Moongkarndi, P., Ongsakul, M., Lamb, A.J., 1980. Impaired local immune response in vitamin A-deficient rats. Clin. Exp. Immunol. 40, 127–135.
Smith, S.M., Levy, N.S., Hayes, C.E., 1987. Impaired immunity in vitamin A-deficient mice. J. Nutr. 117, 857–865.
Spiegl, N., Didichenko, S., McCaffery, P., Langen, H., Dahinden, C.A., 2008. Human basophils activated by mast cell-derived IL-3 express retinaldehyde dehydrogenase-II and produce the immunoregulatory mediator retinoic acid. Blood 112, 3762–3771.
Sun, C.M., Hall, J.A., Blank, R.B., Bouladoux, N., Oukka, M., Mora, J.R., Belkaid, Y., 2007. Small intestine lamina propria dendritic cells promote de novo generation of Foxp3 T reg cells via retinoic acid. J. Exp. Med. 204, 1775–1785.
Svensson, M., Johansson-Lindbom, B., Zapata, F., Jaensson, E., Austenaa, L.M., Blomhoff, R., Agace, W.W., 2008. Retinoic acid receptor signaling levels and antigen dose regulate gut homing receptor expression on CD8+ T cells. Mucosal Immunol. 1, 38–48.
Yokota, A., Takeuchi, H., Maeda, N., Ohoka, Y., Kato, C., Song, S.Y., Iwata, M., 2009. GM-CSF and IL-4 synergistically trigger dendritic cells to acquire retinoic acid-producing capacity. Int. Immunol. 21, 361–377.