Biochemical and Biophysical Research Communications 454 (2014) 537–542

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Retinoids induce integrin-independent lymphocyte adhesion through RAR-α nuclear receptor activity ☆

Jarrett T. Whelan [a], Lei Wang [a], Jianming Chen [a], Meagan E. Metts [a], Taj A. Nasser [a], Liam J. McGoldrick [a], Lance C. Bridges [a,b,*]

[a] Department of Biochemistry and Molecular Biology, The Brody School of Medicine at East Carolina University, Greenville, NC 27834, United States
[b] East Carolina Diabetes and Obesity Institute, The Brody School of Medicine at East Carolina University, Greenville, NC 27834, United States

ARTICLE INFO

Article history:
Received 16 October 2014
Available online 31 October 2014

Keywords:
Retinoid
Disintegrin
Adhesion

ABSTRACT

Oxidative metabolites of vitamin A, in particular all-*trans*-retinoic acid (atRA), have emerged as key factors in immunity by specifying the localization of immune cells to the gut. Although it is appreciated that isomers of retinoic acid activate the retinoic acid receptor (RAR) and retinoid X receptor (RXR) family of nuclear receptors to elicit cellular changes, the molecular details of retinoic acid action remain poorly defined in immune processes. Here we employ a battery of agonists and antagonists to delineate the specific nuclear receptors utilized by retinoids to evoke lymphocyte cell adhesion to ADAM (a disintegrin and metalloprotease) protein family members. We report that RAR agonism is sufficient to promote immune cell adhesion in both immortal and primary immune cells. Interestingly, adhesion occurs independent of integrin function, and mutant studies demonstrate that atRA-induced adhesion to ADAM members required a distinct binding interface(s) as compared to integrin recognition. Anti-inflammatory corticosteroids as well as 1,25-$(OH)_2D_3$, a vitamin D metabolite that prompts immune cell trafficking to the skin, potently inhibited the observed adhesion. Finally, our data establish that induced adhesion was specifically attributable to the RAR-α receptor isotype. The current study provides novel molecular resolution as to which nuclear receptors transduce retinoid exposure into immune cell adhesion.

1. Introduction

Vitamin A (retinol) is vital for the establishment and maintenance of immunity [1]. The requirement of vitamin A for immune function has been appreciated since the early 1900's when it was originally described as an "anti-infective agent" [2–4]. Only within the past decade have the initial molecular details whereby vitamin A contributes to immunity been delineated [1]. Although multiple isomers exist, all-*trans*-retinoic acid (atRA) is the most abundant within human cells and is required for a spectrum of immune-related functions including cellular proliferation and differentiation, antibody production, and cytokine secretion [5–9].

Classically, atRA and other vitamin A derivatives exert their influence by activating two distinct family of nuclear steroid receptors known as the retinoic acid receptor (RAR) and retinoid X receptor (RXR) family [10,11]. Nuclear receptor activation by retinoids, compounds chemically related to vitamin A, prompt well-defined conformational changes that promote the dissociation and association of transcriptional repressors and activators, respectively [12]. The RAR family traditionally pairs with RXR members to form heterodimers with each family exhibiting three main isotypes, designated α, β, γ.

The clinical use of retinoids encompasses immune concerns such as vaccine efficacy and tolerance [13–17]. In addition, the use of atRA in the treatment of acute promyelocytic leukemia is commonly cited as an evolutionary milestone in cancer therapy [18]. Despite their success, adverse side effects that accompany retinoid-based therapies have limited their full potential. Delineating the specific nuclear receptors governing these events is needed for the refinement of retinoid-based therapies.

Since cell adhesion and migration are paramount in immunity, the particular role of retinoids in these processes has been an area of particular interest [19,20]. Seminal studies have established that atRA bestows gut homing properties to lymphocytes [21–23].

Abbreviations: 1,25-$(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; ADAM, a disintegrin and metalloprotease; atRA, all-*trans*-retinoic acid; CHX, cycloheximide; Dex, dexamethasone; RA, retinoic acid; MPSS, methylprednisolone; MAdCAM-1, mucosal addressin cell adhesion molecule-1; RAR, retinoic acid receptor; RXR, retinoid X receptor.

☆ *Funding sources:* This work was supported by start-up funds from the Brody School of Medicine Research and Graduate Studies (LCB).

* Corresponding author at: 115 Heart Drive, East Carolina Heart Institute, Greenville, NC 27834, United States.
E-mail address: bridgesl@ecu.edu (L.C. Bridges).

http://dx.doi.org/10.1016/j.bbrc.2014.10.120
0006-291X/

Induction of α4β7 integrin expression by atRA, promotes gut specific localization via recognition of mucosal addressin cell adhesion molecule-1 (MAdCAM-1) [23]. However, the nuclear receptors required for transducing retinoid exposure into adhesion and migratory changes are not well defined. In addition, immune cell trafficking entails the progressive involvement of multiple protein ligands and receptors. The influence of retinoids in other aspects of adhesion aside from α4β7 and MAdCAM-1 remain unknown.

We recently demonstrated that retinoid exposure promotes cell adhesion through integrin dependent and independent mechanisms in various immune lineages to ADAM (a disintegrin and metalloprotease) members [24,25]. ADAMs are a unique class of integrin ligands that exhibit protease function [26,27]. However, unlike matrix molecules or well-characterized trafficking molecules, the role of ADAMs in cell adhesion and migration is poorly understood. Here we resolve the molecular details of how retinoic acid promotes atypical integrin-independent immune cell adhesion to select ADAM members.

2. Materials and methods

2.1. Cells

Dr. John Wilkins (University of Manitoba) generously provided the RPMI 8866 B cell line. Human peripheral blood mononuclear cells (PBMCs) were purchased from Astarte Biologics (Bellevue, WA). All lymphocytes were maintained at 37 °C with a 5% CO_2 atmosphere in RPMI1640 media containing HEPES, 1% pen/strep, 10% FBS, and 1% sodium pyruvate.

2.2. Reagents

Phosphatase substrate, retinoids, 1,25-$(OH)_2$ vitamin D_3, corticosteroids were all purchased from Sigma (St. Louis, MO). Agonists and antagonists were obtained from TOCRIS bioscience through R&D systems (Minneapolis, MN). Retinoids were dissolved at the desired concentration in DMSO and handled accordingly to minimize light exposure. Antibodies specific for RAR-α and actin were obtain from EMD Millipore (Billerica, MA). The antibodies specific for the RAR-β and γ isotypes were purchased from Santa Cruz Biotechnology Inc. (Dallas, TX). The activating anti-β7 monoclonal antibody 2G3 was the gift of Dr. Ted Yednock (Elan Pharmaceuticals).

2.3. Recombinant ADAM protein ligands

Recombinant ADAM proteins were generated as Fc-fusion proteins in eukaryotic High 5 insect cells as described [28]. The coding regions of the entire disintegrin-like domains of human ADAM9 (Pro^{415}-Asn^{508}), ADAM12 (Asn^{414}-Gly^{519}), ADAM15 (Met^{420}-Gly^{515}), ADAM28 (Thr^{407}-Gly^{500}), and ADAM33 (Ala^{418}-Gly^{510}) were used. For the ADAM28 domain fragments the regions were: ecto (Lys^{22}-His^{667}), ProMet (Lys^{19}-Pro^{408}), and DisEGF (Thr^{407}-His^{664}). The mouse version of ADAM28 (OriGene Technologies Inc., Rockville, MD) was utilized to generate the ecto-Fc construct. Recombinant ligands were evaluated for purity by SDS–PAGE under reducing conditions (supplemental). Charge-to-alanine point mutants of the ADAM28 Dis-Fc protein were generated as previously described [29].

2.4. Static cell adhesion assay

The assay was conducted according to established protocols [24]. Briefly, recombinant proteins were immobilized on Immulon-2 HB microtiter wells (Thermo Scientific, Waltham, MA). Nonspecific adhesion was minimized with 2% (w/v) bovine serum albumin. Cells were cultured for the defined times with the indicated amount of retinoid, vitamin, drug or an equimolar concentration of vehicle (DMSO). Unless otherwise indicated, buffer contained EDTA to eliminate integrin contribution to cell adhesion [30]. RPMI 8866 cells (2×10^5 cells/well) or primary human PBMCs (2.5×10^5 cells/well) were incubated with ligands for 1 h at 37 °C in 5% CO_2. After washing, phosphatase assay buffer was added to wells. After 30 min, color was disclosed with 1 *N* NaOH and an A_{405} reading was obtained. Values obtained with wells coated exclusively with BSA were considered as background values for each experimental condition and were subtracted before reporting final values. Average A_{405} = Average $A_{405(\text{recombinant protein})}$ − Average $A_{405(\text{BSA})}$.

2.5. Western blotting

B cell lysates were prepared in RIPA buffer containing protease inhibitors. A total of 25 μg of cell lysate was analyzed, and antibodies were used as recommended by supplier.

2.6. Statistical analyses

All data were analyzed using GraphPad Prism 6.0. A student's *t*-test was utilized to establish significance. When appropriate for multiple comparisons, a one-way ANOVA analysis with a Tukey's *post hoc* test was employed. Specific *p*-values and thresholds are contained within the figure legends. Error bars represent standard deviation. Unless otherwise indicated, results are representative of at least three independent experiments done in triplicate.

3. Results

3.1. De novo transcription and translation are required for retinoid-induced lymphocyte cell adhesion

Recent discoveries implicate retinoids in extranuclear and nontranscriptional cellular effects, however, this mechanism has not been observed with immune cells [31]. Therefore, we sought to determine if *de novo* transcription and translation are required for retinoids to induce integrin-independent cell adhesion to ADAM28. ADAM28 is involved in leukocyte transmigration and TNF-α release [32,33]. As we previously reported, exposure of RPMI 8866 B cells with atRA induced adhesion to the ADAM28 disintegrin-like domain independent of integrin function in buffer containing EDTA (Fig. 1). A hallmark of integrin function is the requirement of divalent cations for ligand recognition and EDTA is a well-known pan-integrin inhibitor [30]. Cycloheximide (CHX), a well-characterized translational inhibitor, reduced adhesion levels of atRA treated cells to levels obtained with vehicle alone (Fig. 1A). As a functional measure of viability, $MnCl_2$ was added back to the cycloheximide treated cells (Fig. 1A inset). This divalent cation activates integrins and promotes ligand recognition of viable cells. A similar approach described for Fig. 1A was utilized to determine if *de novo* transcription was required for retinoic acid to induce B cell adhesion. The presence of α-amanitin, a well-characterized transcriptional inhibitor, significantly reduced the ability of atRA to induce integrin-independent cell adhesion (Fig. 1B). Again, inclusion of $MnCl_2$ resulted in robust adhesion in the α-amanitin treated cells. Since these data suggest that retinoic acid induces immune cell adhesion through the "classic" genomic mechanism involving RAR/RXR nuclear receptors, we sought to identify the specific nuclear receptor(s) involved.

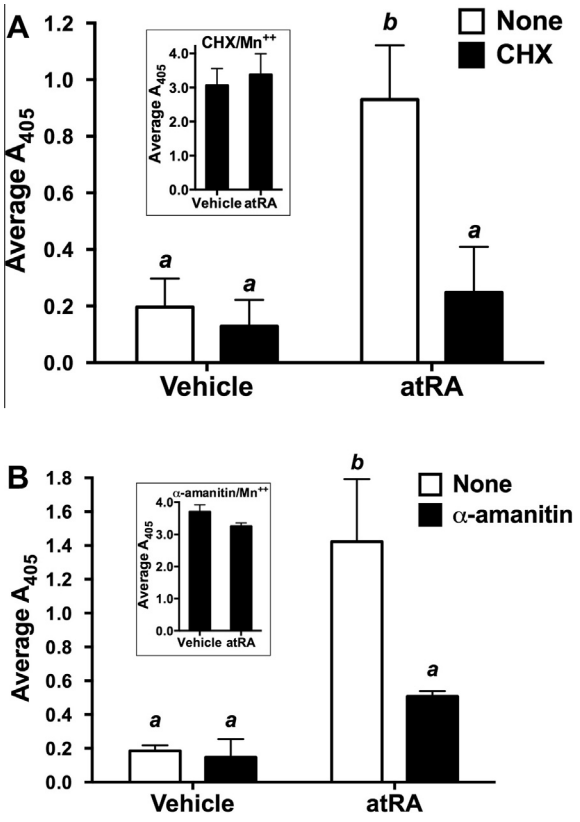

Fig. 1. Retinoic acid stimulated immune cell adhesion requires *de novo* transcription and translation. (A) RPMI 8866 cells were exposed to 1 μM atRA or an equimolar concentration of vehicle for 72 h. Cells were co-cultured in the presence (*black*) or absence (*white*) of 100 nM cycloheximide (CHX). Adhesion assays were conducted as described in experimental procedures on 5 μg/ml of human ADAM28 Dis-Fc. Inset demonstrates that both vehicle and atRA treated cells exposed to CHX are viable and capable of robust adhesion when integrins are activated by the inclusion of $MnCl_2$. (B) Experiments were conduct as described in panel A except with 2 μg/ml of the transcriptional inhibitor α-amanitin. Dissimilar letters denote significant ($p < 0.01$) changes in adhesion.

3.2. RAR activation is sufficient for immune cell adhesion

Pan-RAR or RXR receptor agonists were tested for their ability to induce immune cell adhesion. Although atRA is an RAR exclusive ligand, we have shown that 9-*cis*-RA exposure results in immortal and primary immune B cell adhesion [24]; therefore, an RXR agonist was included in the analysis to address this plausible mode of action. We utilized the RAR pan-agonists TTNPB and RXR pan agonist SR11237 [34–36]. Dosing and exposure time were chosen based upon similar conditions within the primary literature. TTNPB alone was sufficient to induce dose-dependent adhesion trends to ADAM28 that closely mimicked those of atRA (Fig. 2A). Similar results were obtained with another RAR agonist, adapalene (data not shown). These findings indicate that retinoic acid modulates immune cell adhesion through a RAR-containing nuclear receptor.

We wanted to determine if cell adhesion induced by RAR activation was localized to the disintegrin domain of ADAM28. We have previously described production and purification of a recombinant protein fragments encompassing the disintegrin-like, cysteine-rich, and EGF domains (DisEGF), as well as a negative control comprised of the non-adhesive regulatory pro and catalytic metalloprotease regions (ProMet) [28]. The entire ectodomain of ADAM28 was also expressed (Ecto). As evident in Fig. 2B, recombinant proteins containing the disintegrin-like domain facilitated integrin-independent adhesion of retinoid treated RPMI 8866 B cells while the ProMet region did not.

The molecular interface required for integrin recognition of ADAM28 has been extensively mapped with charge-to-alanine mutants [29,37]. Adhesion to ADAM28 disintegrin-like domain mutants was determined with retinoid treated cells in buffer containing EDTA. In addition, cell adhesion in buffer containing divalent cations and the anti-β7 activating monoclonal antibody 2G3 was determined. This approach allowed a direct comparison of the residues comprising the integrin-binding site to those contributing to the retinoid-induced adhesion receptor(s). Multiple lysine residues were required for β7 integrin recognition, a pattern consistent with that established for β1 integrin recognition [29]. In contrast, alanine substitutions of these same lysine residues did not adversely impact adhesion of atRA treated cells (Fig. 2C). The binding interface employed for the integrin-independent adhesion induced by atRA is rather distinct from that required by integrins.

Adhesion selectivity was examined with the disintegrin-like domains of three non-immune ADAMs and the disintegrin-like domain of ADAM33, an ADAM genetically linked to asthma [38]. Although TTNPB exposure induced RPMI 8866 B cell adhesion to all disintegrin domains tested, there was a consistent statistical proclivity for adhesion to the disintegrin domains of the two immune ADAMs, ADAM28 and ADAM33 (Fig. 2D). These data establish that RAR activation is sufficient to prompt adhesion that is selective for immune ligands.

3.3. Corticosteroids and vitamin D metabolites inhibit RAR-induced immune cell adhesion

Adverse effects of RA-based therapies (e.g. differentiation syndrome) are ameliorated upon corticosteroid administration [18]. Apart from the clinical setting, corticosteroids inhibit immune cell–cell interactions, clustering, and adhesion observed upon RA exposure [24,39]. As these observations stem from studies utilizing various retinoids including 9-*cis*-RA, it remains unclear if corticosteroids inhibit an RAR or RXR mediated event. Two distinct corticosteroids, methylprednisolone and dexamethasone, were capable of blunting the adhesion response when RARs were selectively activated in RPMI 8866 cells (Fig. 3A).

The dihydroxy metabolite of vitamin D designated 1,25-$(OH)_2D_3$ biases mobilization of immune cells to the skin niche [1]. Exposure of primary T cells with 1,25-$(OH)_2D_3$ inhibits atRA-mediated integrin receptor expression [21]. Since the adhesion induced with RAR activity in the RPMI 8866 cell line occurs independent of integrins, we explored the role of 1,25-$(OH)_2D_3$ in this novel adhesion response. Co-culturing cells in the presence of the pan RAR agonist TTNPB and increasing concentrations of 1,25-$(OH)_2D_3$ resulted in a dose-dependent inhibition (Fig. 3B). This suggests that 1,25-$(OH)_2D_3$ exposure inhibits multiple aspects of retinoid signaling in immune cell adhesion.

3.4. RAR-α activity transduces retinoid exposure into cell adhesion

To validate the relevance of the RAR activation in promoting immune cell adhesion, primary human PBMCs cultured *ex vivo* in the presence of the RAR specific agonist TTNPB. As shown in Supplementary Fig. 1, a summary of four separate experiments conducted with multiple PBMC donors establishes that primary immune cells cultured in the presence of TTNPB transitioned to integrin-independent adhesion as compared to vehicle treated cells (Fig. 4A). These data demonstrate that immortal and primary immune cells exhibit a novel integrin-independent adhesion response that is mediated by RAR nuclear receptor activity.

Establishing that RAR signaling governs the induction of immune cell adhesion enabled us to employ a battery of RAR isotype-specific agents to further delineate the specific receptor(s) involved. RPMI 8866 B cells were cultured in the presence of

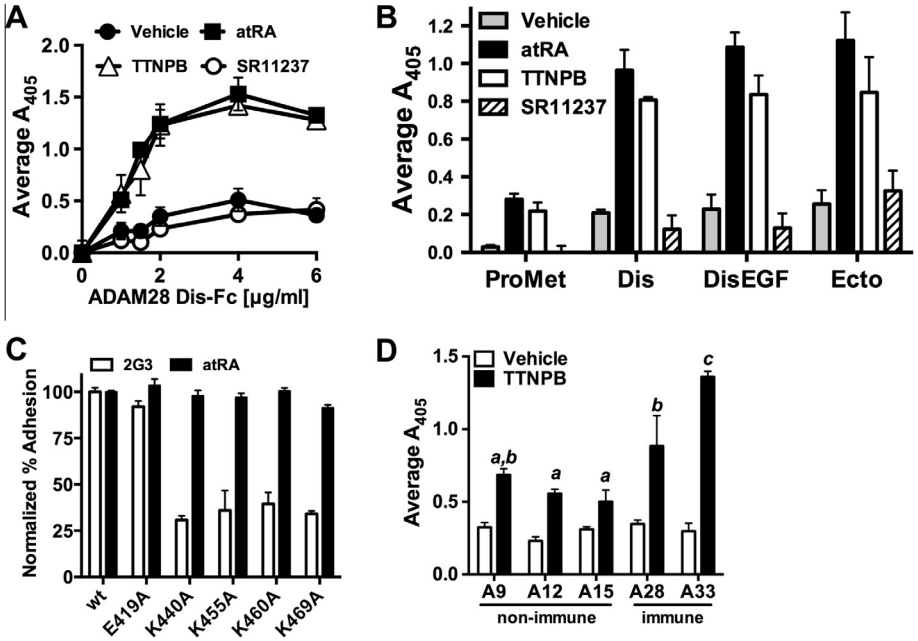

Fig. 2. Immune cell adhesion is prompted with RAR activation. (A) RPMI 8866 cells were cultured for 72 h with vehicle (*filled circles*), 1 μM atRA (*filled squares*), 5 nM of the pan-RAR agonist TTNPB (*open triangles*), or 5 nM of the pan-RXR agonist SR11237 (*open circles*). (B) RPMI 8866 cells exposed to vehicle (*gray*), 0.5 μM atRA (*black*), 5 nM of the pan-RAR agonist TTNPB (*white*), or 5 nM of the pan-RXR agonist SR11237 (*hatched*) for 72 h were evaluated for adhesion to the indicated ligands encompassing various domains of ADAM28 (4 μg/ml). (C) Microtiter wells were coated with various charge-to-alanine mutants of ADAM28 (5 μg/ml). Adhesion assays were repeated as described with RPMI 8866 cells cultured with vehicle or 1 μM atRA for 72 h. The activating anti-β7 monclonal antibody, 2G3, was added for a direct comparison of the interface required for adhesion. Normalized % Adhesion = $\text{Average}A_{405(\text{mutant})}/\text{Average}A_{405(\text{wild type})} \times 100$. (D) Adhesion of RPMI 8866 human B cells exposed to 5 nM TTNPB (*black*) or vehicle (*white*) for 72 h was determined on select ADAM disintegrin domains (4 μg/ml). Dissimilar letters denote significant ($p < 0.05$) changes in adhesion with the TTNPB condition only.

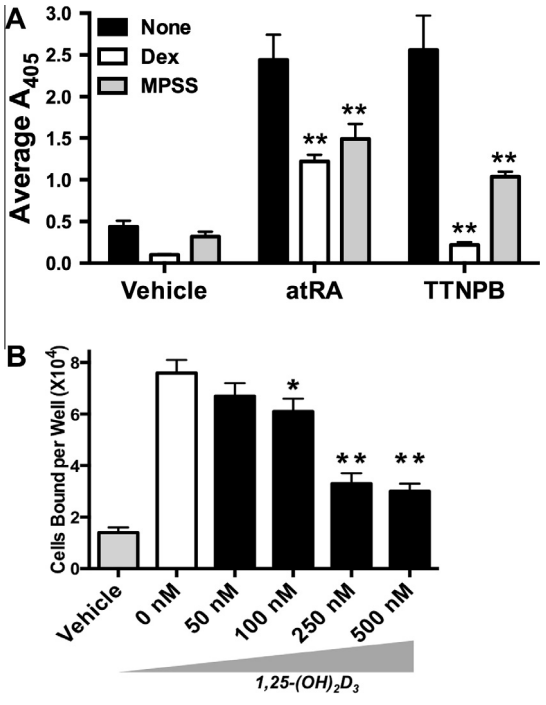

Fig. 3. Retinoid induced immune cell adhesion to ADAM28 is attenuated by corticosteroids or vitamin D metabolites. (A) RPMI 8866 B cells were cultured in the presence of 100 nM atRA, 5 nM of TTNPB or vehicle for 24 h. Cells were administered the corticosteroids, dexamethasone (*white*) and methylprednisolone (*grey*), at 200 μg/ml for 24 h. Double asterisks indicate a significant ($p < 0.01$) decrease of cell adhesion. (B) Adhesion assays were repeated with cells treated with vehicle (*grey*), 5 nM of TTNPB alone (*white*) or in combination of increasing levels of 1,25-$(OH)_2D_3$ (*black*) for 72 h. Asterisks denote a significant decrease in adhesion response when compared to atRA alone ($^*p < 0.05$, $^{**}p < 0.01$).

well-defined RAR-α (AM 580), RAR-β (AC 261066), or RAR-γ (BMS 961) specific agonists. Agonist concentrations were based upon twice the established EC_{50} values [36]. Only the agonist selective for the RAR-α isotype induced RPMI 8866 cell adhesion to the ADAM28 disintegrin-like domain in a manner comparable to atRA (Fig. 4B). We confirmed the results with RAR isotype specific antagonists. As shown in Fig. 4C, only the RAR-α antagonist, ER50891, blocked atRA-induced adhesion as compared to the RAR-β/γ antagonist, CD2665. The observed RAR-α responsiveness was not due to a lack of RAR-β or RAR-γ proteins, as all forms were expressed (Fig. 4D).

4. Discussion

Here we address, in molecular detail, how retinoid exposure induces immune cell adhesion. Adhesion was not mediated by integrin receptors as is traditionally observed with atRA induced cell adhesion [40–42]. Of particular note, RAR but not RXR agonism generated a robust adhesion response. Combining the agonists did not result in augmented levels of adhesion compared to RAR activation alone (data not shown). The induced adhesion was selective and discriminated between multiple ADAM family members. This difference in ligand preference may account for variations in trafficking to aid in recruiting/retaining immune cells to different niches. This concept is further supported since 1,25-$(OH)_2D_3$ inhibited the atRA induced adhesion. Vitamin A and D derivatives have similar roles in immune cell trafficking but are dissimilar in the final locale of immune cells. RAR signaling required for adhesion was also potently blocked by corticosteroids. These findings provide a mechanistic explanation as to how interventions such as corticosteroids likely attenuate RAR signaling to resolve complications associated with retinoid-based therapies.

Refinement and advancement of existing therapies mandate identification of the specific nuclear receptors governing these

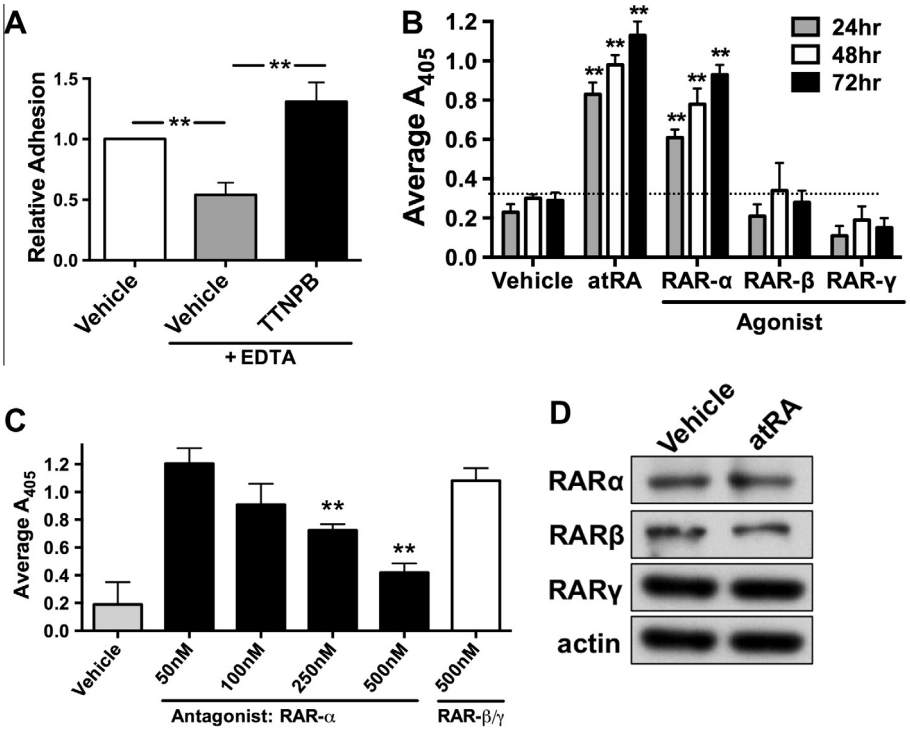

Fig. 4. RAR-α nuclear receptor activity promotes immune cell. (A) Human PBMCs were cultured in the presence of TTNPB (5 nM) or vehicle for 48 h. Abscissa represents data from four different experiments that have been normalized to adhesion levels obtained with vehicle treated PBMCs with functional integrin receptors (no EDTA). (B) RPMI 8866 cells were cultured in the presence of various RAR isotype-specific agonists for 24 (*grey*), 48 (*white*), or 72 (*black*) hours. (C) Antagonists for RAR-α or RAR-β/γ were added to RPMI 8866 cells at the indicated concentrations for 24 h. Cells were then exposed to 50 nM atRA for an additional 24 h before harvesting. (D) Cell lysate (25 μg/lane) of RPMI 8866 cells treated with vehicle or atRA for 72 h were blotted with antibodies specific for RAR isotypes. Actin was detected as a loading control. ($^{**}p < 0.01$).

events. Due to the prevalence and sustained success of retinoids in the clinical setting, delineating how retinoids generate adhesion warrants investigation. Here we establish that the observed immune cell adhesion was attributable to the RAR-α nuclear receptor isotype. It is interesting that this same nuclear receptor is involved with the induction of the gut homing α4β7 integrin, but dissimilar from our findings, α4β7-mediated adhesion additionally required activation of RXR receptors [43]. Perhaps, a distinct subset of nuclear receptors are utilized within immune cells to elicit distinct changes in trafficking receptor expression and function. The finding that the adhesion response evoked by atRA employed a different molecular interface for ligand binding as compared to established receptors lends credence to this concept.

The current work demonstrates, with molecular resolution, how atRA promotes integrin-independent immune cell adhesion. Further work is needed to: (i) clarify and define which specific nuclear receptors participate in other vital aspects of immunity, (ii) establish the mechanisms (e.g. genomic versus non-genomic) by which those particular receptors propagate vitamin A derived signals in immunity, and (iii) identify adhesion receptor(s) induced by retinoids apart from integrins. The importance of this future endeavor is underscored by the historical success of retinoid-based therapies and the undiscovered therapies still embodied within vitamin A.

Acknowledgment

The authors are indebted to Dr. Cheryl Knudson for her thorough reading of the manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.10.120.

References

[1] J.R. Mora, M. Iwata, U.H. von Andrian, Vitamin effects on the immune system: vitamins A and D take centre stage, Nat. Rev. Immunol. 8 (2008) 685–698.
[2] H.N. Green, E. Mellanby, Vitamin a as an anti-infective agent, Br. Med. J. 2 (1928) 691–696.
[3] E. Mellanby, H.N. Green, Vitamin A as an anti-infective agent: its use in the treatment of puerperal septigaemia, Br. Med. J. 1 (1929) 984–986.
[4] R.D. Semba, Vitamin A as "anti-infective" therapy, 1920–1940, J. Nutr. 129 (1999) 783–791.
[5] A. Ertesvag, S. Naderi, H.K. Blomhoff, Regulation of B cell proliferation and differentiation by retinoic acid, Semin. Immunol. 21 (2009) 36–41.
[6] M. Iwata, The roles of retinoic acid in lymphocyte differentiation, Semin. Immunol. 21 (2009) 1.
[7] D.C. Brown, H. Tsuji, R.S. Larson, All-trans retinoic acid regulates adhesion mechanism and transmigration of the acute promyelocytic leukaemia cell line NB-4 under physiologic flow, Br. J. Haematol. 107 (1999) 86–98.
[8] L. Mou, P. Lankford-Turner, M.V. Leander, R.P. Bissonnette, R.M. Donahoe, W. Royal, RXR-induced TNF-alpha suppression is reversed by morphine in activated U937 cells, J. Neuroimmunol. 147 (2004) 99–105.
[9] P.M. Villiger, R. Terkeltaub, M. Lotz, Monocyte chemoattractant protein-1 (MCP-1) expression in human articular cartilage. Induction by peptide regulatory factors and differential effects of dexamethasone and retinoic acid, J. Clin. Investig. 90 (1992) 488–496.
[10] P. Chambon, A decade of molecular biology of retinoic acid receptors, FASEB J. 10 (1996) 940–954.
[11] A. Aranda, A. Pascual, Nuclear hormone receptors and gene expression, Physiol. Rev. 81 (2001) 1269–1304.
[12] A.R. de Lera, W. Bourguet, L. Altucci, H. Gronemeyer, Design of selective nuclear receptor modulators: RAR and RXR as a case study, Nat. Rev. Drug Discovery 6 (2007) 811–820.
[13] J.A. Hall, J.R. Grainger, S.P. Spencer, Y. Belkaid, The role of retinoic acid in tolerance and immunity, Immunity 35 (2011) 13–22.
[14] B. Cassani, E.J. Villablanca, J. De Calisto, S. Wang, J.R. Mora, Vitamin A and immune regulation: role of retinoic acid in gut-associated dendritic cell education, immune protection and tolerance, Mol. Aspects Med. 33 (2012) 63–76.
[15] L.R. Howe, Rexinoids and breast cancer prevention, Clin. Cancer Res. 13 (2007) 5983–5987.
[16] A. Pileri, C. Delfino, V. Grandi, N. Pimpinelli, Role of bexarotene in the treatment of cutaneous T-cell lymphoma: the clinical and immunological sides, Immunotherapy 5 (2013) 427–433.
[17] A.C. Ross, Vitamin A supplementation and retinoic acid treatment in the regulation of antibody responses in vivo, Vitam. Horm. 75 (2007) 197–222.

[18] M.A. Sanz, D. Grimwade, M.S. Tallman, B. Lowenberg, P. Fenaux, E.H. Estey, T. Naoe, E. Lengfelder, T. Buchner, H. Dohner, A.K. Burnett, F. Lo-Coco, Management of acute promyelocytic leukemia: recommendations from an expert panel on behalf of the European LeukemiaNet, Blood 113 (2009) 1875–1891.
[19] T.A. Springer, Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm, Cell 76 (1994) 301–314.
[20] E.C. Butcher, L.J. Picker, Lymphocyte homing and homeostasis, Science 272 (1996) 60–66.
[21] H. Sigmundsdottir, J. Pan, G.F. Debes, C. Alt, A. Habtezion, D. Soler, E.C. Butcher, DCs metabolize sunlight-induced vitamin D3 to 'program' T cell attraction to the epidermal chemokine CCL27, Nat. Immunol. 8 (2007) 285–293.
[22] M. Iwata, Retinoic acid production by intestinal dendritic cells and its role in T-cell trafficking, Semin. Immunol. 21 (2009) 8–13.
[23] M. Iwata, A. Hirakiyama, Y. Eshima, H. Kagechika, C. Kato, S.Y. Song, Retinoic acid imprints gut-homing specificity on T cells, Immunity 21 (2004) 527–538.
[24] J.T. Whelan, J. Chen, J. Miller, R.L. Morrow, J.D. Lingo, K. Merrell, S.R. Shaikh, L.C. Bridges, 9-cis-retinoic acid promotes cell adhesion through integrin dependent and independent mechanisms across immune lineages, J. Nutr. Biochem. 24 (2013) 832–841.
[25] L.C. Bridges, J.D. Lingo, R.A. Grandon, M.D. Kelley, All-trans-retinoic acid induces integrin-independent B-cell adhesion to ADAM disintegrin domains, Biochemistry 47 (2008) 4544–4551.
[26] J.M. White, ADAMs: modulators of cell–cell and cell–matrix interactions, Curr. Opin. Cell Biol. 15 (2003) 598–606.
[27] D.R. Edwards, M.M. Handsley, C.J. Pennington, The ADAM metalloproteinases, Mol. Aspects Med. 29 (2008) 258–289.
[28] L.C. Bridges, P.H. Tani, K.R. Hanson, C.M. Roberts, M.B. Judkins, R.D. Bowditch, The lymphocyte metalloprotease MDC-L (ADAM 28) is a ligand for the integrin alpha4beta1, J. Biol. Chem. 277 (2002) 3784–3792.
[29] L.C. Bridges, K.R. Hanson, P.H. Tani, T. Mather, R.D. Bowditch, Integrin alpha4beta1-dependent adhesion to ADAM 28 (MDC-L) requires an extended surface of the disintegrin domain, Biochemistry 42 (2003) 3734–3741.
[30] K. Zhang, J. Chen, The regulation of integrin function by divalent cations, Cell Adhes. Migration 6 (2012) 20–29.
[31] Z. Al Tanoury, A. Piskunov, C. Rochette-Egly, Vitamin A and retinoid signaling: genomic and nongenomic effects: thematic review series: fat-soluble vitamins: vitamin A, J. Lipid Res. 54 (2013) 1761–1775.
[32] O.J. McGinn, W.R. English, S. Roberts, A. Ager, P. Newham, G. Murphy, Modulation of integrin alpha4beta1 by ADAM28 promotes lymphocyte adhesion and transendothelial migration, Cell Biol. Int. 35 (2011) 1043–1053.
[33] J.B. Jowett, Y. Okada, P.J. Leedman, J.E. Curran, M.P. Johnson, E.K. Moses, H.H. Goring, S. Mochizuki, J. Blangero, L. Stone, H. Allen, C. Mitchell, V.B. Matthews, ADAM28 is elevated in humans with the metabolic syndrome and is a novel sheddase of human tumour necrosis factor-alpha, Immunol. Cell Biol. 90 (2012) 966–973.
[34] M. Mark, N.B. Ghyselinck, P. Chambon, Function of retinoid nuclear receptors: lessons from genetic and pharmacological dissections of the retinoic acid signaling pathway during mouse embryogenesis, Annu. Rev. Pharmacol. Toxicol. 46 (2006) 451–480.
[35] P.F. Egea, A. Mitschler, D. Moras, Molecular recognition of agonist ligands by RXRs, Mol. Endocrinol. 16 (2002) 987–997.
[36] A.R. Moise, Pharmacology of retinoid receptors, Tocris Rev. 36 (2011) 1–11.
[37] L.C. Bridges, D. Sheppard, R.D. Bowditch, ADAM disintegrin-like domain recognition by the lymphocyte integrins alpha4beta1 and alpha4beta7, Biochem. J. 387 (2005) 101–108.
[38] S.D. Shapiro, C.A. Owen, ADAM-33 surfaces as an asthma gene, N. Engl. J. Med. 347 (2002) 936–938.
[39] R.S. Larson, D.C. Brown, L.A. Sklar, Retinoic acid induces aggregation of the acute promyelocytic leukemia cell line NB-4 by utilization of LFA-1 and ICAM-2, Blood 90 (1997) 2747–2756.
[40] M.M. Medhora, Retinoic acid upregulates beta(1)-integrin in vascular smooth muscle cells and alters adhesion to fibronectin, Am. J. Physiol. Heart Circ. Physiol. 279 (2000) H382–387.
[41] M.M. Escribese, E. Conde, A. Martin, D. Saenz-Morales, D. Sancho, G. Perez de Lema, J. Lucio-Cazana, F. Sanchez-Madrid, M.L. Garcia-Bermejo, F.M. Mampaso, Therapeutic effect of all-trans-retinoic acid (at-RA) on an autoimmune nephritis experimental model: role of the VLA-4 integrin, BMC Nephrol. 8 (2007) 3.
[42] A.M. Hanson, J. Gambill, V. Phomakay, C.T. Staten, M.D. Kelley, 9-cis-retinoic acid and troglitazone impacts cellular adhesion, proliferation, and integrin expression in K562 cells, PLoS ONE 9 (2014) e93005.
[43] H. Takeuchi, A. Yokota, Y. Ohoka, H. Kagechika, C. Kato, S.Y. Song, M. Iwata, Efficient induction of CCR9 on T cells requires coactivation of retinoic acid receptors and retinoid X receptors (RXRs): exaggerated T cell homing to the intestine by RXR activation with organotins, J. Immunol. 185 (2010) 5289–5299.