

B-cells and Inflammation in the Absence of the Abelson Related Gene (*Arg*)

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Abstract

The Abelson non-receptor tyrosine kinases, c-Abl and Arg, are important regulators of cellular processes in cancer, inflammation, infection, and neuronal dynamics. Recent research on the role for these kinases in processes involving interactions with the cytoskeleton or signaling molecules, may lead to further insight into the pathogenesis of a variety of disorders, including chronic inflammatory diseases. In a mouse model for multiple sclerosis, we recently reported that Arg deficient mice develop T-cell mediated autoimmune neuro-inflammation with the same severity as littermate controls, but display a different B-cell phenotype upon immunization. Here we comment on these results and discuss the role for Arg in B-cell activation and homeostasis.

Keywords: Arg/Abl2; c-Abl; Experimental autoimmune encephalomyelitis (EAE); B-cells

Introduction

The v-abl Abelson murine leukemia viral oncogene 2 (*Abl2*), or Abelson related gene (*Arg*), encodes a non-receptor tyrosine Abl kinase, which together with c-Abl (Abl1) comprises the family of Abl tyrosine kinases [1]. These tyrosine kinases play important roles in the regulation of the cytoskeleton, formation of membrane vesicles, transmission of signals for proliferation and survival of cells, and for downstream signaling through both the T- and B-cell receptors [2-5]. Recent research within this field has revealed new insight into the importance of the Abl enzymes in the context of solid tumors, neuronal structure, infection, and inflammation [5-8]. In particular, the role for Arg in dynamics of the cytoskeleton has opened new ways to the understanding of spreading of tumor metastases [8,9].

The Arg protein has two C-terminal F-actin binding domains and one microtubule-binding domain, allowing for the crosslinking of F-actin and microtubules in cytoskeletal rearrangements [10]. The importance of Arg in the control of cytoskeletal activity is demonstrated upon binding of integrins on the cell surface and the following downstream molecular interactions. Ligation of integrin induces the phosphorylation of the p190RhoGAP protein by Arg, subsequently leading to inhibition of the RhoGTPase RhoA, which results in reduced cell contractility and cell migration [11-13].

In T lymphocytes, antigen recognition by the T-cell receptor induces activation of Abl via the Src family tyrosine kinase Lck [3]. Activation of the Abl kinases through the T-cell receptor subsequently induces phosphorylation of the signaling molecules Zap-70 and linker of activated T-cells (LAT) [3]. Furthermore, in Abl/Arg deficient T-cells, decreased activation of PLC γ 1, Shc, Jnk and NF- κ B was demonstrated [2]. Taken together, these studies show an important role for the Abl/Arg kinases in T-cell activation.

In a recent article [14], we described the role for Arg in development of Myelin Oligodendrocyte Glycoprotein (MOG35-55)-induced, T-cell dependent experimental autoimmune encephalomyelitis (EAE), a widely used experimental model for multiple sclerosis (MS). We showed that lack of Arg does not change the course of disease compared to disease development in Arg sufficient mice. The result was somewhat unexpected because of the previously demonstrated role for the Abl kinases in T-cell signaling and function [15], and we hypothesized that T lymphocyte activation, and thereby the autoimmune response, would be affected in the MOG35-55 immunized *Arg*^{-/-} mice. In line with the EAE data, *in vitro* stimulation of T cells from the Arg deficient mice did not show an altered response compared to the *Arg*^{+/+} littermates [14]. We therefore concluded that the lack of Arg does not influence T cell activation, neither by MOG(35-55) peptide, mycobacteria, or Pertussis Toxin, which are all included in the induction of EAE, nor by activation of T lymphocytes *in vitro*. One explanation would be that the Arg deficiency is compensated for by the activity of c-Abl.

Similar to the T-lymphocyte activation *in vitro*, stimulation of B-cells with anti-IgM antibodies or LPS was not affected by lack of the Arg kinase. However, when investigating the B cell population in spleen from MOG(35-55) immunized *Arg*^{-/-} and *Arg*^{+/+} mice, we observed a significant decrease in the relative numbers of B cells in the immunized Arg deficient compared to the non-immunized mice [14]. A number of studies have shown that the Abl kinases are important players in the humoral immune system [16-18]. Reports on a distinct role for the Arg tyrosine kinase in B-cell development, homeostasis, and function are, however, limited in the literature.

The role for Abl kinases in B-cell activation

How could the decreased B cell number in the spleen of the *Arg*^{-/-} mice, upon immunization, be explained? B-cells develop in the bone marrow where they pass through a number of checkpoints to become immune-competent cells, ready to pass into the populations of B1 cells in the lung and epithelia, and into conventional B2 cells, or marginal

zone B cells, in the spleen [19]. From studies of c-Abl deficient mice, a marked reducing effect on development of early, non-mature B-cells was observed [16, 20], suggesting that *Arg* cannot make up for the effects of c-Abl deficiency in developing B-cells. Similar observations regarding B cell development have not been reported for *Arg* deficient mice; instead it has been shown that the highest level of *Arg* expression is found in mature B-cells [17]. In fact, spleen has been shown to be one of the organs with the highest levels of *Arg* expression [21]. This might lead to the assumption that lack of *Arg* in mature B-cells would give an observable effect upon stimulation of *Arg*-deficient spleen cells *ex vivo*. As mentioned above, our data, however, showed that the *in vitro* proliferative response upon stimulation with anti-IgM or LPS was the same in *Arg* deficient and *Arg* sufficient mice [14]. This is in contrast to c-Abl knockout mice, where reduced cell activation and response to anti-IgM in stimulated B-cells was reported [18]. Moreover, a reduction in the number of peritoneal B-1 cells was observed in the *Abl1^{-/-}* mice [4]. In the same study, it was demonstrated that c-Abl is interacting with the B-cell co-receptor CD19 and, thus, clearly has a role in B-cell activation [4]. Results showing c-Abl aggregating with the B-cell Fc-receptor, FcγRIIB1, furthermore suggest that c-Abl is important in Fc-receptor mediated apoptosis [22].

Similar to the B-cell response in the *Arg* deficient mice, the T-cell response *in vitro* was not affected in the absence of *Arg* [14]. Taken together, these and previously reported data suggest that any functional consequences due to the absence of the *Arg* kinase in B- and T-lymphocyte activation, are rescued by the presence of c-Abl. The remaining question is whether the reduced relative number of B-cells in spleen of immunized *Arg^{-/-}* is one phenotype that cannot be fully compensated for by c-Abl?

B- and T-cell dependent induction of EAE in the context of *Arg*

In our study of EAE development in *Arg^{-/-}* mice, the MOG(35-55) peptide was used for immunization and induction of CNS inflammation [14]. It has been argued that short myelin peptides do not induce a complex immune response, dependent on both T- and B-cells and more resembling the complexity in MS, but rather models a pure T-cell response, circumventing the B-cell recognition of large conformational epitopes [23,24]. Moreover, short peptides could bind directly to MHC molecules for presentation, leaving aside a role for B-cells as antigen presenting cells [25]. One could argue that if the disease development we observe as a result of MOG(35-55) immunization in the *Arg^{-/-}* mice is due to c-Abl rescuing a T-cell response, induction of EAE with whole MOG protein might be more interactive with signaling pathways involving *Arg*. This would make it possible to elucidate whether *Arg* has a role in B-cell signaling or migration, in a complex inflammatory disease.

We recently studied congenic mice, carrying a genetic fragment, including the *Arg* gene, from a genetically different mouse strain, the EAE resistant RIIS/J mouse strain, on a black strain background. From sequence analyses we found coding polymorphisms in the *Arg* gene when comparing the two mouse strains, and immunization with myelin peptides revealed that the congenic strain developed significantly reduced clinical EAE (Jacobsen, F.A. et al, to be published elsewhere). In order to further analyze the inflammatory response in mice with genetic variation in *Arg*, and to investigate EAE induction including a B-cell dependent response, congenic and littermate C57Bl/6 mice were immunized with rMOG protein [26]. The results

showed that mice with variant alleles all developed EAE upon immunization with rMOG; the congenic mice with higher incidence of disease and slightly, but not significantly, more severe disease (Table 1). These data, together with the results from the *Arg^{-/-}* mice, might suggest that genetic variation in *Arg* results in a differential response when EAE is induced with peptide versus with whole myelin protein. However, when *Arg* is absent, the mice develop as severe EAE as their *Arg* sufficient littermates upon immunization with peptide.

	Mouse strain ^a	
Disease phenotype ^b	C57Bl/6_Eae27 ^{B6} (n=10)	C57Bl/6_Eae27 ^{RIIS/J} (n=9)
Incidence	50%	89%
Mean Max Score	1.8 ± 2.1 ^c	2.9 ± 1.7
Mean day of onset	16.7 ± 6.8	19.1 ± 4.9

^aThe *Eae27* locus [34] comprising a 4 Megabasepair (Mbp) polymorphic region from the RIIS/J strain was bred to the C57Bl/6 background and littermate offspring was generated by heterozygous breeding. Mice with one or two B6 alleles in *Eae27* were grouped, since disease development was the same in the two groups. ^bMice were immunized with 100 µg rMOG (a kind gift from Prof. Trevor Owens, University of Southern Denmark) in Complete Freund's Adjuvans (Difco) and 2x200 ng of Pertussis Toxin (SigmaAldrich). Scoring of EAE was previously described [5]. Mean Max Score is the mean of the highest individual score in each group. Mean day of onset is the first day with a score >1. ^cSD

Table 1: EAE development in mice with variant *Arg* alleles upon immunization with rMOG.

Possible molecular interactions in the regulation of B-cells by *Arg*

Additional cellular processes involving the action of the *Arg* tyrosine kinase could be investigated for the influence on B-cell homeostasis and function. In order for lymphocytes to migrate during development, upon infection, and immune stimulation, they express integrins that can interact with adhesion molecules on endothelial cells. Integrins are heterodimers composed of one α and one β chain that interact with components of the extracellular matrix, transducing signals to the cytoskeleton. A number of α and β chain subunits exist, and the variants are combined to form a large number of integrin molecules with specific distribution and function [27]. Through advanced studies of fibronectin adhesion, time-lapse video recordings, and phosphorylation experiments applying fibroblasts expressing *Arg*-mutants, it has been shown that interaction between the β-1 integrin and *Arg* leads to functional effects in the cytoskeleton, including cell migration and cellular protrusion [13,28]. *Arg* was recently demonstrated to directly interact with the β-1 integrin, which results in activation of the enzyme [29].

Furthermore, the importance of β-1 integrins in cell migration was demonstrated in experiments where β-1 integrin deficient cells in chimeric mice, did not colonize the spleen [30]. Could the lack of *Arg*, and, consequently, a lack of interaction between *Arg* and β-1 integrin, thus, lead to a reduced expansion and lower migration rate of mature, antigen activated B cells into the spleen, in the MOG35-55 immunized *Arg* deficient mice?

B-cells have, in contrast to being disease promoting, also been implicated as regulators of the inflammatory response in EAE [31,32].

Regulatory B-cells have primarily been described as IL-10 producing, having an inhibitory effect on inflammation-promoting processes like antigen presentation, expression of co-stimulatory molecules, and production of pro-inflammatory cytokines [33]. These cells have, in addition, been shown to induce the differentiation of regulatory T cells, a property suggested to be dependent on the expression of glucocorticoid-induced TNFR family-related protein (GITR) [34]. Regulatory B cells, in addition, produce TGF- β . In co-culturing studies, it was shown that LPS-stimulated B-cells, but not B-cells stimulated through other signaling pathways, had an inhibitory effect on T-cell activation, which could be reversed by anti-TGF- β antibodies [35,36]. In an OVA induced allergy model, a population of regulatory, CD5⁺, TGF- β producing B-cells were shown to induce Foxp3 expression in CD4⁺ T-cells, resulting in the suppression of allergic airway disease [37]. Conversely, it has been reported that TGF- β 1 has a strong inhibitory effect on B-cells. Upon exposure *in vitro*, TGF- β 1 induced apoptosis in resting human B-cells [38]. Furthermore, studies on human tonsillar B-cells, stimulated with *Staphylococcus aureus in vitro*, showed down-regulation of surface immunoglobulin (Ig) expression and secretion upon treatment [39]. It should be noted that the observations from the abovementioned studies, demonstrating TGF- β production by activated B-cells versus B-cells responding to TGF- β through suppression of cellular activities, are based on differential experimental systems. The studies on inhibition of T-cell activation were performed with LPS stimulation of mouse B-lymphocytes, while the studies on B-cell inhibition by TGF- β were done with human resting cells or cells stimulated with bacteria not expressing LPS. However, taken together these studies demonstrate that TGF- β 1 is an important regulator in B-cell biology. In a recent study of a model for high glucose levels and renal fibrosis, it was demonstrated that *Arg* expression is down-regulated in the presence of glucose-induced high levels of TGF- β 1 [40]. Moreover, suppression of *Arg* expression by the tyrosine-kinase inhibitor Imatinib, lead to increased expression of TGF- β 1. Interestingly, TGF- β 1, among other factors, is induced by reactive oxygen species (ROS), which in turn increases ubiquitylation and subsequent degradation of *Arg* [40,41]. It remains to be elucidated whether TGF- β 1, negatively acting on B-cells, is increased in the absence of *Arg*, and whether that could explain parts of the reduced number of B-cells observed in the immunized *Arg*^{-/-} mice [14].

Conclusion

In this commentary we have discussed and raised some questions concerning the role for the *Arg* tyrosine kinase in B-cell responses and autoimmune inflammation. We speculate that: (-) in the absence of *Arg*, Abl-dependent, T-cell mediated autoimmunity is carried out by c-Abl; (-) the lower relative B-cell number in spleen of immunized *Arg*^{-/-} mice could partly be a result of the absence of interaction between β 1-integrin and *Arg* and/or to an increased expression of TGF- β 1, due to the lack of *Arg*, having a reducing effect on the number of mature B cells; (-) genetic variation in *Arg* influences peptide-induced EAE, but not B-cell dependent induction of EAE with whole myelin protein. We suggest that the issues raised should be investigated in order to further elucidate the role for the *Arg* tyrosine kinase as a drug target in inflammatory diseases.

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References

1. Pendergast AM (2002) The Abl family kinases: mechanisms of regulation and signaling. *Adv Cancer Res* 85: 51-100.
2. Gu JJ, Zhang N, He YW, Koleske AJ, Pendergast AM (2007) Defective T cell development and function in the absence of Abelson kinases. *J Immunol* 179: 7334-7343.
3. Zipfel PA, Zhang W, Quiroz M, Pendergast AM (2004) Requirement for Abl kinases in T cell receptor signaling. *Curr Biol* 14: 1222-1231.
4. Zipfel PA, Grove M, Blackburn K, Fujimoto M, Tedder TF, et al. (2000) The c-Abl tyrosine kinase is regulated downstream of the B cell antigen receptor and interacts with CD19. *J Immunol* 165: 6872-6879.
5. Khatri A, Wang J, Pendergast AM (2016) Multifunctional Abl kinases in health and disease. *J Cell Sci* 129: 9-16.
6. Greuber EK, Smith-Pearson P, Wang J, Pendergast AM (2013) Role of ABL family kinases in cancer: from leukaemia to solid tumours. *Nat Rev Cancer* 13: 559-571.
7. Kerrisk ME, Koleske AJ (2013) *Arg* kinase signaling in dendrite and synapse stabilization pathways: memory, cocaine sensitivity, and stress. *Int J Biochem Cell Biol* 45: 2496-2500.
8. Wang J, Pendergast AM (2015) The Emerging Role of ABL Kinases in Solid Tumors. *Trends Cancer* 1: 110-123.
9. Gil-Henn H, Patsialou A, Wang Y, Warren MS, Condelis JS, et al. (2013) *Arg*/Abl2 promotes invasion and attenuates proliferation of breast cancer in vivo. *Oncogene* 32: 2622-2630.
10. Miller A L, Wang Y, Mooseker M S, Koleske A J (2004) The Abl-related gene (*Arg*) requires its F-actin-microtubule cross-linking activity to regulate lamellipodial dynamics during fibroblast adhesion. *J Cell Biol* 165: 407-419.
11. Hernández SE, Settleman J, Koleske AJ (2004) Adhesion-dependent regulation of p190RhoGAP in the developing brain by the Abl-related gene tyrosine kinase. *Curr Biol* 14: 691-696.
12. Bradley WD, Hernández SE, Settleman J, Koleske AJ (2006) Integrin signaling through *Arg* activates p190RhoGAP by promoting its binding to p120RasGAP and recruitment to the membrane. *Mol Biol Cell* 17: 4827-4836.
13. Peacock J G, Miller A L, Bradley W D, Rodriguez O C, Webb D J, et al. (2007) The Abl-related gene tyrosine kinase acts through p190RhoGAP to inhibit actomyosin contractility and regulate focal adhesion dynamics upon adhesion to fibronectin. *Mol Biol Cell* 18: 3860-3872.
14. Jacobsen F A, Hulst C, Bäckström T, Koleske A J, Andersson Å (2016) *Arg* deficiency does not influence the course of myelin oligodendrocyte glycoprotein (MOG35-55)-induced Experimental Autoimmune Encephalomyelitis. *J Clin Cell Immunol* 7: 420.
15. Gu JJ, Ryu JR, Pendergast AM (2009) Abl tyrosine kinases in T-cell signaling. *Immunol Rev* 228: 170-183.
16. Hardin JD, Boast S, Schwartzberg PL, Lee G, Alt FW, et al. (1995) Bone marrow B lymphocyte development in c-abl-deficient mice. *Cell Immunol* 165: 44-54.
17. Bianchi C, Muradore I, Corizzato M, Cornacchini G, Beretta L, et al. (2002) The expression of the non-receptor tyrosine kinases *Arg* and c-abl is differentially modulated in B lymphoid cells at different stages of differentiation. *FEBS letters* 527: 216-222.
18. Liberatore RA, Goff SP (2009) c-Abl-deficient mice exhibit reduced numbers of peritoneal B-1 cells and defects in BCR-induced B cell activation. *Int Immunol* 21: 403-414.
19. Melchers F (2015) Checkpoints that control B cell development. *J Clin Invest* 125: 2203-2210.
20. Schwartzberg PL, Stall AM, Hardin JD, Bowdish KS, Humaran T, et al. (1991) Mice homozygous for the *abl*1 mutation show poor viability and depletion of selected B and T cell populations. *Cell* 65: 1165-1175.

21. Koleske AJ, Gifford AM, Scott ML, Nee M, Bronson RT, et al. (1998) Essential roles for the Abl and Arg tyrosine kinases in neurulation. *Neuron* 21: 1259-1272.
22. Tzeng SJ, Bolland S, Inabe K, Kurosaki T, Pierce SK (2005) The B cell inhibitory Fc receptor triggers apoptosis by a novel c-Abl family kinase-dependent pathway. *J Biol Chem* 280: 35247-35254.
23. Lyons JA, San M, Happ MP, Cross AH (1999) B cells are critical to induction of experimental allergic encephalomyelitis by protein but not by a short encephalitogenic peptide. *Eur J Immunol* 29: 3432-3439.
24. Lyons JA, Ramsbottom MJ, Cross AH (2002) Critical role of antigen-specific antibody in experimental autoimmune encephalomyelitis induced by recombinant myelin oligodendrocyte glycoprotein. *Eur J Immunol* 32: 1905-1913.
25. Davoust J, Banchereau J (2000) Naked antigen-presenting molecules on dendritic cells. *Nat Cell Biol* 2: E46-48.
26. Adelmann M, Wood J, Benzl I, Fiori P, Lassmann H, et al. (1995) The N-terminal domain of the myelin oligodendrocyte glycoprotein (MOG) induces acute demyelinating experimental autoimmune encephalomyelitis in the Lewis rat. *J Neuroimmunol* 63: 17-27.
27. Iwamoto DV, Calderwood DA (2015) Regulation of integrin-mediated adhesions. *Curr Opin Cell Biol* 36: 41-47.
28. Lapetina S, Mader CC, Machida K, Mayer BJ, Koleske AJ (2009) Arg interacts with cortactin to promote adhesion-dependent cell edge protrusion. *J Cell Biol* 185: 503-519.
29. Simpson MA, Bradley WD, Harburger D, Parsons M, Calderwood DA, et al. (2015) Direct interactions with the integrin β 1 cytoplasmic tail activate the Abl2/Arg kinase. *J Biol Chem* 290: 8360-8372.
30. Fässler R, Meyer M (1995) Consequences of lack of beta 1 integrin gene expression in mice. *Genes Dev* 9: 1896-1908.
31. Wolf SD, Dittel BN, Hardardottir F, Janeway CA Jr (1996) Experimental autoimmune encephalomyelitis induction in genetically B cell-deficient mice. *J Exp Med* 184: 2271-2278.
32. Ray A, Wang L, Dittel BN (2015) IL-10-independent regulatory B-cell subsets and mechanisms of action. *Int Immunol* 27: 531-536.
33. Pestka S, Krause CD, Sarkar D, Walter MR, Shi Y, et al. (2004) Interleukin-10 and related cytokines and receptors. *Annu Rev Immunol* 22: 929-979.
34. Ray A, Basu S, Williams CB, Salzman NH, Dittel BN (2012) A novel IL-10-independent regulatory role for B cells in suppressing autoimmunity by maintenance of regulatory T cells via GITR ligand. *J Immunol* 188: 3188-3198.
35. Tian J, Zekzer D, Hanssen L, Lu Y, Olcott A, et al. (2001) Lipopolysaccharide-activated B cells down-regulate Th1 immunity and prevent autoimmune diabetes in nonobese diabetic mice. *J Immunol* 167: 1081-1089.
36. Parekh VV, Prasad DV, Banerjee PP, Joshi BN, Kumar A, et al. (2003) B cells activated by lipopolysaccharide, but not by anti-Ig and anti-CD40 antibody, induce anergy in CD8+ T cells: role of TGF-beta 1. *J Immunol* 170: 5897-5911.
37. Natarajan P, Singh A, McNamara JT, Secor ER, Jr, Guernsey LA, et al. (2012) Regulatory B cells from hilar lymph nodes of tolerant mice in a murine model of allergic airway disease are CD5+, express TGF-beta, and co-localize with CD4+Foxp3+ T cells. *Mucosal Immunol* 5: 691-701.
38. Lomo J, Blomhoff HK, Beiske K, Stokke T, Smeland EB (1995) TGF-beta 1 and cyclic AMP promote apoptosis in resting human B lymphocytes. *J Immunol* 154: 1634-1643.
39. Kehrl JH, Thevenin C, Rieckmann P, Fauci AS (1991) Transforming growth factor-beta suppresses human B lymphocyte Ig production by inhibiting synthesis and the switch from the membrane form to the secreted form of Ig mRNA. *J Immunol* 146: 4016-4023.
40. Torsello B, Bianchi C, Meregalli C, Di Stefano V, Invernizzi L, et al. (2016) Arg tyrosine kinase modulates TGF-beta1 production in human renal tubular cells under high-glucose conditions. *J Cell Sci* 129: 2925-2936.
41. Cao C, Li Y, Leng Y, Li P, Ma Q, et al. (2005) Ubiquitination and degradation of the Arg tyrosine kinase is regulated by oxidative stress. *Oncogene* 24: 2433-2440.