Journal of Clinical & Cellular Immunology

Andersson and Jacobsen, J Clin Cell Immunol
2016, 7:6
DOI: 10.4172/2155-9899.1000470

Commentary OMICS International

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

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Received date: September 07, 2016; **Accepted date:** November 11, 2016; **Published date:** November 18, 2016

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 PLCg1, Shc, Jnk and NF-kB 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 RIIIS/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$^{RIIIS/J}$ (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

[a]The Eae27 locus [34] comprising a 4 Megabasepair (Mbp) polymorphic region from the RIIIS/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. [b]Mice were immunized with 100 µg rMOG (a kind gift from Prof. Trevor Owens, University of Southern Denmark) in Complete Freunds 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. [c]SD

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 ß chain that interact with components of the extracellular matrix, transducing signals to the cytoskeleton. A number of α and ß 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 ß-1 intergrin 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 ß-1 integrin, which results in activation of the enzyme [29].

Furthermore, the importance of ß-1 integrins in cell migration was demonstrated in experiments where ß-1 intergrin 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 ß-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-ß. 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-ß antibodies [35,36]. In an OVA induced allergy model, a population of regulatory, $CD5^+$, TGF-ß 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-ß1 has a strong inhibitory effect on B-cells. Upon exposure *in vitro*, TGF-ß1 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-ß production by activated B-cells versus B-cells responding to TGF-ß 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-ß were done with human resting cells or cells stimulated with bacteria not expressing LPS. However, taken together these studies demonstrate that TGF-ß1 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-ß1 [40]. Moreover, suppression of Arg expression by the tyrosine-kinase inhibitor Imatinib, lead to increased expression of TGF-ß1. Interestingly, TGF-ß1, 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-ß1, 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 ß1-integrin and Arg and/or to an increased expression of TGF-ß1, 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.

Acknowledgement

We wish to thank Mathilde Caldara for taking care of the animal facility. This work was supported by Novo Nordisk, Denmark, The Warwara Larsen Foundation, The Carlsberg Foundation, The Karen A Tolstrup Foundation, and The A.P. Møller Foundation.

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