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Optimal T Cell Activation and B Cell Antibody Responses In Vivo Require the Interaction between Leukocyte Function–Associated Antigen-1 and Kindlin-3

Vicky Louise Morrison,*1 Liisa M. Uotila,*1 Marc Llort Asens,* Terhi Savinko,* and Susanna Carola Fagerholm*1‡

Kindlin-3 is an important integrin regulator that is mutated in the rare genetic disorder, leukocyte adhesion deficiency type III, a disorder characterized by defective neutrophil trafficking and platelet function, leading to recurrent bacterial infections and bleeding. Kindlin-3 is also known to regulate T cell adhesion in vitro and trafficking in vivo, but whether the integrin/kindlin interaction regulates T or B cell activation in vivo is unclear. In this study, we used TTT/AAA β2-integrin knock-in (KI) mice and TCR-transgenic (OT-II) KI mice, in which the integrin/kindlin connection is disrupted, to investigate the role of the integrin/kindlin interaction in T cell activation. We show that basal T cell activation status in these animals in vivo is normal, but they display reduced T cell activation by wild-type Ag-loaded dendritic cells in vitro. In addition, T cell activation in vivo is reduced. We also show that basal Ab levels are normal in TTT/AAA but they display reduced T cell activation by wild-type Ag-loaded dendritic cells in vitro. In conclusion, we show that the integrin/kindlin interaction is required for trafficking of immune cells, as well as for T cell activation and B cell Ab responses in vivo. These results imply that the immunodeficiency found in leukocyte adhesion deficiency type III patients, in addition to being caused by defects in neutrophil function, may be due, in part, to defects in lymphocyte trafficking and activation. The Journal of Immunology, 2015, 195: 000–000.

Integrins are heterodimeric cell surface adhesion molecules consisting of α and β subunits. Expression of the β2 (CD18) integrin subfamily is restricted to leukocytes, where they play important roles in cellular adhesion and migration in the immune system (1). The β2-integrin family member leukocyte function–associated Ag-1 (LFA-1; αLβ2, CD11a/CD18) is highly expressed in lymphocytes, namely B and T cells, and mediates binding to ICAMs on the surface of other cells. LFA-1 mediates firm adhesion to endothelial cells, which is necessary for extravasation of lymphocytes from the bloodstream into lymph nodes and sites of inflammation (2). Indeed, LFA-1–knockout mice display impaired lymphocyte homing to these sites (3–6).

In addition to its role in cellular migration, LFA-1 is a key component of the immunological synapse (IS) that forms between immune cells. For example, IS formation between APCs, such as dendritic cells (DCs), and CD4 T cells initiates T cell activation, and subsequent IS formation between CD4 T cells and B cells allows the provision of help for B cell Ab production. Specifically, LFA-1 forms part of the peripheral supramolecular activation cluster that surrounds the TCR or BCR cluster in the center, thus stabilizing the synapse and ensuring efficient lymphocyte activation (7). It is thought that LFA-1 downstream signaling may also contribute to the cellular activation signals, thus performing a co-stimulatory function, as was shown in T cells (8–10). However, the involvement of LFA-1 in T and B cell activation in vivo remains controversial because of the difficulty in segregating the roles of LFA-1 in migration versus activation.

Conformational changes in LFA-1 required for optimal ligand binding, as well as downstream integrin signaling, are regulated by the binding of cytoplasmic factors to the integrin subunit intracellular domains. Previously, we generated a mutant mouse line in which the threonine triplet in the β2-integrin tail was substituted with alanine residues (TTT/AAA β2-integrin knock-in (KI) mouse). This mutation abolishes binding of the important integrin regulator kindlin-3 to the integrin cytoplasmic domain (11), resulting in impaired integrin activation to its high-affinity state and, therefore, reduced integrin function. We showed that polyclonal activation of TTT/AAA β2-integrin KI T cells in vitro with soluble anti-CD3 results in a reduction in T cell activation and proliferation compared with wild-type (WT) T cells, whereas activation in response to plate-bound anti-CD3 is unaffected by the KI mutation (11). Ag-specific T and B cell activation in vivo in these mice remains largely unexplored.

Using integrin TTT/AAA β2-integrin KI and KI TCR-transgenic (OT-II) mouse models, we show that Ag-specific CD4 T cell activation in both an in vitro coculture system with DCs and in vivo in the spleen is dependent on the β2-integrin TTT site. We also reveal that optimal B cell numbers in lymph nodes and B cell Ab responses in vivo are dependent on fully functioning LFA-1 in leukocytes. These results indicate a vital role for LFA-1–mediated firm adhesion

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The online version of this article contains supplemental material.

Abbreviations used in this article: BMDC, bone marrow–derived dendritic cell; DC, dendritic cell; Fo, follicular; IS, immunological synapse; KI, knock-in; LFA-1, leukocyte function–associated Ag-1; MZ, marginal zone; WT, wild-type.

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in lymphocyte activation, even in circumstances where LFA-1-mediated migration is unlikely to be involved.

Materials and Methods

Mice

Mice were bred and maintained at the University of Dundee or the University of Helsinki, in compliance with national and local rules. The TTT/AAA β2-integrin KI mouse line was described previously (11). The KI mice were crossed with OT-II mice (provided by Prof. Colin Watts, University of Dundee) to generate homozygous Igβ2 KI mice expressing the OT-II transgene. Expression of the v-α2 and v-β8 TCR subunits was monitored by flow cytometry, and the KI mutation as confirmed by PCR.

Bone marrow–derived dendritic cells (BMDCs) were generated by culturing mouse bone marrow for 10 d in 10 ng/ml GM-CSF (PeproTech) in non-tissue culture–treated petri dishes. Cells were given fresh medium and growth factor on days 3, 6, and 8 and were harvested on day 10 using 4 mM EDTA solution. CD4 T cells were purified from mouse spleens and lymph nodes by positive selection using magnetic beads to CD4 (Miltenyi Biotec), according to the manufacturer’s instructions. Splenic B cells were purified by negative selection with anti-mouse CD43 magnetic beads (Miltenyi Biotec), according to the manufacturer’s instructions. The purity of B and T cells was determined by flow cytometry and was typically >95%.

Cell preparation

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ELISAs

Cytokine levels produced in T cell cultures, namely IL-2 and IFN-γ, were measured by ELISA using paired Ab kits (eBioscience), according to the manufacturer’s instructions. Total and OVA-specific serum Ab levels were detected by ELISA. Briefly, 5 µg/ml protein OVA (InvivoGen) was coated onto Nunc MaxiSorp plates at 4 °C overnight. Alternatively, for total Ab measurements, unlabeled Ig subclass–specific Abs (Southern Biotech) were used. After washing, serum was added, and serial dilutions were performed and incubated at room temperature for 2 h. Ab subclass–specific AP–labeled Abs (Southern Biotech) were used for detection, according to standard protocols, and A405 was measured.

Western blot

T and B cells were activated with anti-CD3 or anti-IgM, respectively, for 0, 5, 30, or 60 min and lysed in 1% TX-100, 150 mM NaCl, 50 mM Tris (pH 7.4), 10 mM EDTA in the presence of phosphatase and protease inhibitors (Pierce), and lysates were analyzed by Western blotting. Primary Abs against phospho-Syk/Zap70, total Syk/Zap, phospho-Erk1/2, phospho-Akt308, and total Akt were from Cell Signaling Technology.

Static adhesion assays

Static adhesion assays were performed as described (11). Briefly, the integrin ligands ICAM-1 (6 µg/ml; R&D Systems), fibronectin (10 µg/ml), and VCAM (6 µg/ml; R&D Systems) were coated onto 96-well MaxiSorp plates (Nunc) by overnight incubation at 4 °C. Purified splenic B or T cells (1.5–2 × 10^6 cells/ml) were resuspended in adhesion medium (RPMI 1640 supplemented with 0.1% BSA, 40 mM HEPES, and 2 mM MgCl2) and added to the plate. Where appropriate, cells were stimulated with 200 nM PdBu (Sigma-Aldrich), 10 µg/ml anti-BCR (R&D Systems), 10 µg/ml anti-CD3 (R&D Systems), 0.2 µg/ml LPS (Sigma-Aldrich), or 5 µg/ml MgCl2 + 1 mM EGTA immediately before being added to the plate. Cells were allowed to adhere for 30 min at 37 °C before gentle washing and detection, as described (12).

Results

Impaired activation of KI T cells in vitro

To investigate the role of the β2-integrin TTT site in immune cell activation and function, we made use of a novel TTT/AAA β2-integrin KI mouse that we described recently (11). These mice have a mutated TTT site in the β2-integrin subunit cytoplasmic domain, meaning that the binding of the important integrin regulator, kindlin-3, is lost, and downstream integrin signaling is ablated (11). We reported previously that activation of KI T cells with plate-bound anti-CD3 or with phorbol ester in vitro appears normal, but there is a reduction in T cell activation (expression of CD69 and CD25) and a delay in T cell proliferation when soluble anti-CD3, in conjunction with IL-2, is used as a stimulus (11). We...
show in this article that increasing the amount of soluble anti-CD3 (Fig. 1A, 1B) or IL-2 (Fig. 1C) did not rescue the defect in T cell activation with soluble anti-CD3 seen in the Ki cells. Higher doses of anti-CD3 also did not have any effect on T cell activation (data not shown). Early TCR signaling (p-Zap-70, p-Erk) appeared normal in the Ki T cells (Fig. 1D). However, there was a reduction in Ki T cell aggregation in response to anti-CD3 (Fig. 1E), indicating that cell–cell contact mediated, presumably, by LFA-1 and ICAM-1 on the surface of the T cells was reduced, which may influence T cell activation in this system. Indeed, addition of LFA-1 blocking Ab to the WT T cell cultures reduced T cell activation, showing that this process was LFA-1 dependent (Fig. 1F). Therefore, under conditions in which LFA-1 is engaged with ICAM-1 (using soluble anti-CD3 as a stimulus), polyclonal activation of Ki T cells is impaired, and this impairment cannot be overcome by using increasing amounts of anti-CD3 or IL-2 in the cultures.

Baseline in vivo T cell activation is normal in TTT/AAA β2-integrin Ki mice

Because in vitro Ki T cell activation with soluble anti-CD3 and IL-2 was reduced, we next investigated T cell activation in vivo. Other investigators identified a role for LFA-1 in providing a costimulatory signal to T cells and contributing to their activation in vivo (8–10). Conversely, in the absence of β2-integrins, an increase in baseline T cell activation was reported (13), suggesting a role for β2-integrins in limiting T cell activation. We found that expression of activation markers CD25, CD44, and CD62L by splenic CD4 T cells was normal in unimmunized Ki mice (Fig. 2A). Expression of CD69 was reduced significantly in Ki CD4 T cells, although the expression of CD69 was normal in unimmunized KI mice (Fig. 2A). Therefore, under conditions in which LFA-1 is engaged with ICAM-1 (using soluble anti-CD3 as a stimulus), polyclonal activation of Ki T cells is impaired, and this impairment cannot be overcome by using increasing amounts of anti-CD3 or IL-2 in the cultures.

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FIGURE 1. Impaired activation of TTT/AAA β2-integrin KI T cells in vitro. Polyclonal T cells from WT and KI mice were cultured with various concentrations of soluble anti-CD3 for 24 h; the expression of the activation markers CD69 and CD25 was assessed by flow cytometry (A), and IL-2 production was quantified by ELISA (B) (n = 4 mice). (C) Polyclonal WT and KI T cells were cultured with anti-CD3 (0.167 µg/ml) plus various concentrations of IL-2 for up to 2 d, and expression of CD69 and CD25 was assessed (n = 3 mice). The differences between WT and KI values are statistically significant (p < 0.05) for all data points with the exception of day 0. (D) WT and KI T cell signaling in response to anti-CD3 stimulation was assessed by Western blotting. Data are representative of n = 3 mice. (E) Representative images of T cell cultures showing cell clustering after 24 h of culture with various amounts of anti-CD3 and IL-2. Scale bars, 400 µm. (F) Polyclonal T cells from WT and KI mice were cultured for 24 h in the presence of soluble anti-CD3 (2.5 µg/ml), with or without blocking LFA-1 Ab (15 µg/ml), or were left untreated. The expression of CD69 and CD25 was assessed by flow cytometry (n = 4 mice). All error bars represent SEM. *p < 0.05, **p < 0.01, ***p < 0.001, Student t test.
WT controls (Fig. 5B). Similarly, IgM levels were lower in KI mice from as early as 7 d postimmunization until 3 wk post-immunization, when IgM levels began to diminish (Fig. 5B). These data suggest that optimal Ab production is dependent on the β₂-integrin subunit TTT site in leukocytes.

Because Ab responses were altered in TTT/AAA β₂-integrin KI mice (Fig. 5B), we next investigated B cell development and function in these mice. KI mice had normal proportions of mature (IgD⁺IgM⁺) and immature (IgD⁻IgM⁺) B cells in the bone marrow (Fig. 6A), indicating normal B cell development. However, KI mice displayed abnormal proportions of these cells in the blood; KI mice had less mature and more immature circulating B cells compared with WT mice (Fig. 6B). We also studied follicular (Fo) and marginal zone (MZ) B cell subsets in WT and KI spleens. Flow cytometry analyses showed that the proportions of Fo (CD21intCD23hi) and MZ (CD21hiCD23lo) B cells were normal in the TTT/AAA β₂-integrin KI mouse spleens (Fig. 6C).

We reported previously that KI mice have enlarged spleens, but smaller inguinal and mesenteric lymph nodes, compared with WT mice (11). In this study, we found that the absolute number of B220⁺ B cells was lower in KI lymph nodes than in WT lymph nodes (Fig. 6D), similar to what we reported previously for CD4⁺ T cells (11) and likely reflecting a problem with lymphocyte homing to the lymph nodes in KI mice. B cell numbers in the spleen were slightly increased in KI mice (Fig. 6D) due to splenomegaly. However, the proportions of B cells, as assessed by flow cytometry of isolated cells from spleen and other lymphoid organs (inguinal and mesenteric lymph nodes, Peyer’s patches, and bone marrow), were largely normal in TTT/AAA β₂-integrin KI mice (Fig. 6D). The maturation state of WT and KI B cells in the lymph nodes also was assessed by flow cytometry; despite the reduced cellularity of the lymph nodes, the proportions of mature and immature B cells were normal in KI mice (Fig. 6E).

Together, these data implicate that B cell development in the bone marrow and subsequent maturation in secondary lymphoid organs are not disturbed by the TTT/AAA mutation of β₂-integrin, but problems in homing to lymph nodes result in lower numbers of B220⁺ cells in lymph nodes and an increased proportion of immature B cells in the bloodstream of TTT/AAA β₂-integrin KI mice.

**B cell activation in vitro is normal in TTT/AAA β₂-integrin KI mice**

β₂-integrins were shown to lower the threshold for B cell activation (16), but it is unknown whether LFA-1/kindlin-3 interactions affect B cell activation. To investigate whether the lowered Ag-specific Ab response of KI mice is solely due to impaired T cell activation or whether the mutation also causes changes in B cell function, we studied the functions of WT and KI B cells in more detail. Splenic B cells were characterized for their integrin expression and expression of activation markers. As reported previously for other cell types, there were reduced levels of CD11a and CD18 in KI B cells compared with WT cells, but activation markers were expressed at similar levels in both populations (Supplemental Fig. 2). Static adhesion assays confirmed that TTT/AAA β₂-integrin KI B cells were deficient in binding to ICAM-1 (Fig. 7A), as we reported previously for CD4⁺ T cells (11). Furthermore, KI B cells show less aggregation in response to BCR or LPS stimulus (Fig. 7B). In contrast, KI B cell adhesion to the β₁ integrin ligands fibronectin and VCAM was normal (Supplemental Fig. 3). We next studied the proliferation and activation of splenic B cells in vitro. B cells isolated from WT and KI spleens were grown for 48 h in the presence of anti-IgM or LPS to trigger activation through the BCR and TLR4, respectively. No changes in B cell proliferation (Fig. 7C) or the expression of CD69, CD86, or MHC Class II after 48 h of stimulation were detected (Fig. 7D). Early BCR signaling (p-Syk, p-Akt, p-Erk) also appeared normal in KI cells compared with WT cells (Fig. 7E).

In conclusion, our results show that, although present in normal proportions in peripheral lymphoid organs, B cells lacking the...
β₂-integrin TTT site have problems in homing to the appropriate lymphoid organs and/or out of them. In addition, KI B cells display reduced adhesion in vitro, as well as impaired T cell–dependent and T cell–independent Ab production in vivo. Therefore, the integrin/kindlin-3 interaction is required for optimal T cell activation, B cell homing, and Ab responses in vivo.

**Discussion**

We showed previously that the TTT site in the β₂-integrin subunit is only necessary for T cell activation with soluble anti-CD3 and not with plate-bound anti-CD3 in vitro (11). We now show that this site is essential for the Ag-specific activation and proliferation of T cells in vitro by DCs and in vivo in the spleen following immunization of mice, as well as for optimal Ab responses in vivo. The results from this study highlight that lymphocyte LFA-1 downstream signaling mediated by the TTT site on the β₂-integrin subunit is necessary for optimal immune responses in vivo.

This TTT site in the β₂-integrin cytoplasmic domain regulates integrin function in cells by mediating binding to cytoplasmic proteins, including kindlin-3 (11). Kindlins provide a physical link between the integrin and the cortical actin cytoskeleton and are thought to be involved in integrin conformational change to the active, fully open state, as well as contributing to downstream integrin signaling (17). In lymphocytes, kindlin-3 binds LFA-1 and is essential for firm adhesion of both T and B cells (18–20), and it stabilizes integrin/ligand interactions in T cells following TCR engagement in vitro (21). Meanwhile, 14-3-3 proteins bind...
to the TTT site when one or more of the T residues are phosphorylated (e.g., after T cell activation by phorbol esters) and initiate downstream integrin signaling (22). We showed that mutation of the TTT site in T cells results in the loss of actin reorganization, cell spreading, and adhesion to ICAM-1 (11, 22, 23). Sorting nexin family members also bind this region of the $\beta_2$-integrin tail, specifically in the endocytic compartment during the integrin-recycling process (24), and loss of this association in TTT/AAA KI cells results in reduced expression of integrins on the cell surface, although this reduced expression is unlikely to contribute to the impairments in lymphocyte activation seen in this study (11).

LFA-1 is a key component of the IS that forms between CD4 T cells and DCs or B cells, contributing to the stability of the contacts (7). A loss of LFA-1 function in T cells may reduce the duration of cellular contacts and, thus, have a negative impact on T cell activation. Indeed T cell–DC contact duration, frequency, and intensity have a significant impact on the magnitude and polarization of the T cell response (25). LFA-1 is localized to the peripheral supramolecular activation cluster of the IS and initiates “outside-in” integrin signaling following adhesion to ICAM-1, which, in turn, triggers a range of downstream effects, including further LFA-1 clustering, actin cytoskeleton rearrangement, and orientation of the cell toward the synapse (reviewed in Ref. 26). Thus, it seems likely that integrin signaling relies on TTT-dependent interactions with kindlin-3 and/or 14-3-3 proteins to drive IS formation and subsequent T or B cell activation. Further studies are required to determine the phosphorylation status of the TTT residues under these activation conditions, the specific roles of kindlin-3 and 14-3-3 proteins in this process, and to directly visualize the effects of the TTT/AAA mutation in T cells on IS formation with DCs and B cells. In addition to its role in IS formation, LFA-1 is thought to contribute directly to T cell activation via adhesion-independent mechanisms. For example, previous findings support a role for LFA-1 in initiating and/or maintaining T cell IL-2 production (27) by affecting IL-2 expression at the transcription level (28, 29). LFA-1 also has the potential to impact on the Th1/Th2 balance of the CD4 T cell response (30, 31). In this study, we show that baseline expression of activation markers by splenic T cells was normal in TTT/AAA KI mice and that the proportions of naive and effector/memory T cells in the spleen were unaltered, indicating that the development of these effector/memory responses is not dependent on high-affinity LFA-1.

The specific role for LFA-1 in the lymphocyte-activation process has proved controversial because of the difficulty in dissecting out the involvement of this molecule in lymphocyte homing compared with a direct role in cellular activation. To overcome this, we made use of in vitro–activation techniques. Previously, we showed largely normal activation of KI CD4 T cells in vitro under conditions of polyclonal stimulation with plate-bound anti-CD3 (11). However, when cells are stimulated with soluble anti-CD3, which, in contrast to plate-bound anti-CD3 promotes LFA-1–mediated T cell–T cell synapse formation, T cell activation is significantly reduced (11). In this study, we show a reduction in KI T cell activation in a coculture system with DCs, indicating that in circumstances in which T cell activation requires IS formation with

FIGURE 4. TTT/AAA $\beta_2$-integrin KI T cell activation is impaired in vivo. Purified CD4$^+$ WT or KI OT-II T cells were labeled with CFSE and injected i.v. into the tail vein of recipient WT mice. Twenty-four hours later, recipient mice were immunized i.v. with OVA peptide Ag in LPS adjuvant. Five days postimmunization, spleens were harvested. The percentage of donor CFSE$^+$ cells (A), mean fluorescence intensity of CFSE (B), and expression of the typical T cell activation marker CD44 (C) were measured by flow cytometry ($n = 5$ mice). All error bars represent SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, Student t test.

FIGURE 5. TTT/AAA $\beta_2$-integrin KI mice have impaired Ab responses in vivo. (A) Baseline circulating Ab levels were measured in unimmunized WT and KI mice ($n = 4$ mice). (B) WT and KI mice were immunized s.c. with OVA protein Ag in CFA. Blood samples were taken weekly for 5 wk postimmunization, and circulating Ag-specific Ab levels were measured by ELISA ($n = 5$ mice). All error bars represent SEM. *$p < 0.05$, ***$p < 0.001$, WT versus KI, two-way ANOVA.
APCs (or other T cells), T cell LFA-1 is required for optimal activation (32).

Many previous in vivo studies in β2- or αL-knockout mice reported reduced lymphocyte activation in immune reactions at peripheral sites, such as delayed-type hypersensitivity responses in the footpad (4), contact sensitivity reactions in the ear (3, 4), and peripheral antitumor responses (6), in which LFA-1 likely plays a major role in cellular homing. β2-Integrin involvement in systemic immunity remains disputed, with reports of normal β2-deficient T cell responses to systemic Listeria infection (33) but impaired systemic responses to Streptococcus (3). In this study, to more thoroughly investigate the involvement of the β2-integrin (and specifically the cytoplasmic domain TTT site) in cellular activation rather than migration, we specifically analyzed the T cell response in the spleen, because T cell migration to this organ is unaffected by the TTT/AAA mutation (11).
FIGURE 7. TTT/AAA β₂-integrin KI B cells show impaired adhesion but normal activation and proliferation. (A) Static adhesion assays of splenic B cells purified from WT and KI mice to ICAM-1. Data are from two/three independent experiments (n = 3 mice). (B) B cell aggregation in response to proliferation stimuli. Photomicrographs were taken after 48 h of stimulation with anti-IgM and LPS and are a representative of four independent experiments (n = 6 mice). Scale bar, 400 μm. (C and D) Proliferation and activation of WT and KI splenic B cells in vitro in response to anti-IgM and LPS. CFSE-labeled B cells were grown in the presence of different concentrations of anti-IgM or LPS and subjected to flow cytometry for proliferation (C) and activation (D) after 48 h. Result of three independent experiments (n = 3–5 mice). (E) B cell signaling. Phosphorylation of Syk, Akt, and Erk in WT and KI B cells stimulated through the BCR, with anti-IgM for the indicated times, was determined by Western blot analysis. All error bars represent SEM. *p < 0.05, Student t test.
Analysis of β2-deficient T cell responses in an Ag-specific manner was performed previously using TCR-transgenic β2-knockout mice. However, these studies focused on the gut immune response for which a defect in T cell homing was also shown (34, 35), although the investigators also reported a defect in systemic T cell responses to model Ag (34). In this study, we provide firm evidence to indicate an essential role for fully functioning LFA-1 and, specifically, for β2-integrin tail interactions, in splenic CD4 T cell activation and proliferation in vivo under conditions in which T cell homing does not have an impact. These data build on our previous study in which we showed normal TTT/AAA β2-integrin KI T cell activation in the spleen of KI mice following the adoptive transfer of Ag-loaded DCs (11). The discrepancy between these findings is likely due to the influence of β2-integrin deficiency in other leukocyte populations on the resulting T cell response in our previous study in the global TTT/AAA β2-integrin KI mouse (11) (Supplemental Table I). In addition to their adhesive functions, it is well established that β2-integrins perform regulatory functions, restricting various inflammatory responses of DCs and macrophages (14, 36–38) and limiting subsequent T cell activation (14, 39, 40). In support of this, we show in this study that the splenic environment of KI mice is more inflammatory and suggest that the higher levels of IL-1β in the KI spleen may have contributed to the T cell–activation response observed in our previous study (11, 15).

The role of talin1, another integrin activator that binds to the β2 cytoplasmic domain, also was studied in T cells (32, 41). Talin1 is essential for homing to lymph nodes, as well as in contact-dependent, but not in contact-independent (CD3/CD28- or PMA/ionomycin-induced), T cell proliferation. Talin1 was also critical for prolonged T cell–DC contacts, but the formation of transient contacts was unaffected. The clustering of LFA-1 at the IS was not affected by talin1 deficiency, but the polarization of vinculin and F-actin at the IS was talin1 dependent (32). These and other studies (42) show that kindlin-3 and talin have distinct, although somewhat overlapping, functions in integrin-dependent leukocyte activation, proliferation, and homing to lymphoid organs. It is possible that the integrin/kindlin interaction regulates, for example, integrin clustering at the IS, because kindlin was reported to regulate β1-integrin clustering on the cell surface (43). Our findings are in accordance with these studies. However, both kindlin-3 and talin bind to and activate several integrin families (β1, β2, and β3) (28, 44), whereas our results show the importance of specific β2-integrin interactions with kindlin-3. Furthermore, talin1 does not bind to the TTT/AAA sequence (11).

Previous studies of the Ab levels in unimmunized β2-integrin-knockout mice showed a substantial increase in total Ig and especially in IgG levels present in the bloodstream (3). However, our results show that TTT/AAA β2-integrin KI mice have normal serum Ab levels and that, in response to immunization, Ab production in KI mice is significantly reduced compared with WT mice. B cell functions were studied earlier in mice in whom talin1 was selectively depleted (CD19Tn1–/–) mice (45), revealing the role of active VLA-4 and LFA-1 integrins in B cell functions. Interestingly, the talin1-null B cells show similar Ab-production profile as those from TTT/AAA β2-integrin KI mice, with normal levels of serum Igs in unimmunized mice and severely attenuated levels of IgG and IgM in response to immunization (45). Also, B cell numbers in the lymph nodes were decreased, reflecting the difficulty of talin1-null B cells to home to lymph nodes (45). Because talin1 regulates both LFA-1 and VLA-4 integrins, which are known to have overlapping functions in B cell homing and other functions (46, 47), it is important to note that the TTT/AAA β2-integrin KI mutation alone causes a severe reduction in the Ab response, showing the importance of β2-integrins and their intra-cellular interactions with kindlin and other cytoplasmic-binding partners in Ab production. Specifically, KI mice had a severe impairment in the initial production of IgM, whereas the reduction in IgG levels was more modest, suggesting that the T-independent IgM response might be more dependent on high-affinity LFA-1 than the subsequent T-dependent isotype switch and affinity-maturation steps in B lymphocytes.

Although the defect in Ab production following immunization in KI mice may not be due to attenuated B cell proliferation or proximal BCR signaling, there are several other possible explanations for this result. First, as discussed earlier, KI CD4 T cells show an attenuated ability to form an IS with DCs and to be activated. Because the subsequent formation of an IS between a CD4 T cell and a B cell is vital for the Ab production by the latter, the lack of an appropriate T cell stimulus most likely affects full activation of B cells and optimal Ab production. Second, lymphocyte homing to lymphoid organs, especially lymph nodes, and egress from the circulation are impaired in KI mice, likely affecting B cell interactions with other leukocytes that are required for optimal B cell activation and Ig production. Thus, integrin-mediated interactions with endothelial cells and other leukocytes requiring high-affinity LFA-1 are essential for optimal B cell responses in vivo.

Therefore, our current findings provide novel evidence for an important role of LFA-1–mediated adhesion and/or downstream signaling via the β2 subunit TTT site in optimal systemic T and B cell responses in vivo.

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Disclosures

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References


