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Anti-CD83 promotes IgG1 isotype switch in marginal zone B cells in response to TI-2 antigen

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ABSTRACT

CD83 is a transmembrane glycoprotein that is rapidly up-regulated on activated B cells. Although CD83 itself is incapable to transduce intracellular signaling, it acts as a negative regulator of B cell function. We have recently described that a single application of anti-CD83 antibody results in dramatically enhanced production of antigen-specific IgG1 but not other isotypes upon immunization of mice with the TI-2 model antigen (Ag) NIP-Ficoll. This effect was mediated by the binding of anti-CD83 to CD83 on the surface of B cells themselves. In the current study we show that administration of anti-CD83 enhances IgG1-production independent of IL-4. Application of anti-CD83 does not alter the proliferation and general expansion of NIP-specific B cells. In the presence of anti-CD83, immunized mice develop normal frequencies of plasmablasts in response to NIP-Ficoll of which an increased number produces IgG1. These cells localize in extrafollicular foci in the spleen of immunized mice and originate from the marginal zone B cell pool. Taken together, our results indicate that CD83 engagement *in vivo* does not generally enhance B cell activation but selectively promotes IgG1 class switch in marginal zone B cells in response to TI-2 Ag.

Introduction

CD83 is a highly conserved type-I transmembrane glycoprotein that belongs to the Ig superfamily CD83 is strongly expressed on activated murine and human dendritic cells (DCs) (Berchtold et al., 1999; Zhou and Tedder, 1995) and was long considered a highly specific maturation marker for DCs. Meanwhile CD83 is also known as a marker for activated B cells since B lymphocytes are the predominant CD83-expressing cell population in immune responses to infection (Breloer et al., 2007) and upon vaccination (Prazma et al., 2007). In addition, CD83 is expressed on epithelial cells in the thymus where it is essential for the development of single CD4$^+$ thymocytes and peripheral CD4$^+$ T cells (Fujimoto et al., 2002; Garcia-Martinez et al., 2004).

Apart from its influence on CD4$^+$ T cell development and DC activation (Bates et al., 2014), CD83 is involved in the regulation of B cell maturation and homeostasis (Luthje et al., 2008) as well as in B cell activation and function (Breloer et al., 2007; Kretschmer et al., 2007, 2009). CD83-transgenic (CD83tg) mice display normal T cell responses but respond to immunization with TD or TI model Ags, as well as in infection models, with dramatically reduced immunoglobulin (Ig) production *in vivo* (Breloer et al., 2007). These effects are due to CD83 overexpression on the B cells themselves rather than on accessory cells. Wild-type B cells adoptively transferred into CD83tg mice produce normal levels of Ig upon immunization (Breloer et al., 2007). Furthermore, in mixed bone marrow chimeras only CD83tg B cells do not respond to immunization (Breloer et al., 2007). In addition, B cells from CD83tg animals react to BCR engagement *in vitro* with reduced proliferation (Kretschmer et al., 2007; Uhde et al., 2013) and reduced Ig production upon stimulation with LPS (Kretschmer et al., 2007). These data demonstrate that CD83 acts as a negative regulator of B cell activation. Thereby CD83 interferes with early BCR-mediated signaling

Abbreviations: Ab, antibody; Ag, antigen; Ig, immunoglobulin; CD83tg, CD83-transgenic; FO B cell, follicular B cell; MZ B cell, marginal zone B cell; TI, thymus-independent.

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events such as phosphorylation of LYN and SYK, consequently leading to reduced activation of distal effectors such as ERK1/2 (Uhde et al., 2013) and Ca^{2+} influx (Kretschmer et al., 2007). How CD83 mediates its inhibitory effects is still enigmatic. The short cytoplasmic tail of the CD83 molecule does not contain any known signaling motifs. Therefore, CD83 is not capable to deliver intracellular signals itself but must interact with other signaling molecules. A ligand for CD83, however, has not been identified so far. CD83 ligands (CD83L) are assumed to be present on the cell surface of thymocytes (Fujimoto et al., 2002; Garcia-Martinez et al., 2004), human DCs (Lechmann et al., 2001), monocytes (Scholler et al., 2001) and $CD8^+$ T cells (Scholler et al., 2001; Hirano et al., 2006).

Interestingly, a single injection of a CD83-specific antibody and simultaneous engagement of the BCR by TI-2 model Ag in C57BL/6 mice leads to a 100-fold increase in antigen-specific IgG1 response whereas other isotypes were not affected (Kretschmer et al., 2009). These findings demonstrate a regulatory role of activation-induced CD83 which is rapidly up-regulated on B cells and present on the cell surface within 3 h after activation *in vitro* (Kretschmer et al., 2007) and *in vivo* (Breloer et al., 2007). Anti-CD83 mediates its biologic effect by engaging CD83 on the B cells themselves as it does not affect Ig production in mixed bone marrow chimeras in which selectively the B cells lack CD83 (Kretschmer et al., 2009). Moreover, it has to be present in the very onset of B cell response as administration of anti-CD83 two days after immunization does not enhance Ig production anymore (Kretschmer et al., 2009). It is not yet clear whether anti-CD83 acts as a neutralizing or as an agonistic antibody. As CD83 is not capable to transduce intracellular signals itself, the most likely explanation for the mode of action of anti-CD83 is the blocking of negative signals by interference with the interaction of CD83 with potential regulatory ligand(s).

Two possible mechanisms could be envisaged: (i) Neutralization of negative signals by anti-CD83 could generally enhance the proliferation, survival and expansion of activated B cells including IgG1 producers, or (ii) directly promote IgG1 class switch which would result in enhanced frequencies exclusively of IgG1-producing B cells. The current study was conducted to clarify these questions.

We show that administration of anti-CD83 does not change the number or proliferative response of Ag-specific plasmablasts that are generated upon immunization with TI-2 Ag but clearly elevates the frequency of IgG1-producing plasmablasts. Histological analysis showed that these IgG1 producers are found at the border of B and T cell areas early after immunization and later localize in extrafollicular foci. IgG1-producing cells are negative for IgD and do not express markers characteristic for follicular or germinal center B cells. These cells originate from the MZ B cell pool that is known to respond to TI-2 Ag.

Taken together, our observations strongly suggest that engagement of CD83 *in vivo* by anti-CD83 supports IgG1 class switch in MZ B cells in response to TI-2 Ag independent of IL-4 receptor alpha signaling.

Materials and methods

Mice

C57BL/6, JHT (Gu et al., 1993), BALB/c and BALB/c IL-4 receptor alpha knockout (IL-4R$\alpha^{-/-}$) mice (Mohrs et al., 1999) were bred and maintained at the Bernhard Nocht Institute for Tropical Medicine. NP-specific B cell receptor knock-in mice that have been generated by the replacement of Ig heavy chain D and J elements with a recombined antibody heavy chain variable region derived from a NP-specific antibody (Sonoda et al., 1997) were crossed to kappa-light chain knockout mice (Zou et al., 1993). B cells from the resulting B1-8i mice that express the transgene in combination with endogenous λ1 light chain are NP-specific. Therefore, high numbers of B cells from B1-8i mice respond to NP-haptenated protein or NP-Ficoll with the production of Ag-specific antibodies. B1-8i mice were obtained from the animal facility of the Robert Koch Institute, Berlin, Germany. Mice were used at the age of 6–10 weeks.

Purification and transfer of splenic B1-8i B cells

Single cell suspensions of spleen cells were prepared from B1-8i mice by mashing spleens through cell sieves, followed by erythrocyte lysis with ACK lysis buffer (Invitrogen, Karlsruhe, Germany). B cells were purified from spleens by magnetic cell sorting employing a B cell negative isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's instructions. Purity of the resulting cell populations was verified by flow cytometry to be >98% (data not shown). For Fig. 2 (CD83 expression kinetics), 5 (flow cytometric analysis of $IgG1^+$ plasmablasts) and 6 (histology) 1×10^6 purified B1-8i B cells were injected intravenously (i.v.) into age-matched C57BL/6 recipients. For Figs. 4 and 5, 2×10^6 or 4×10^6 purified B1-8i B cells were injected intraperitoneally (i.p.) into JHT or C57BL/6 recipients in 200 μl PBS.

Purification and transfer of B1-8i marginal zone (MZ) and follicular (FO) B cells

B cells were first enriched from spleens of B1-8i mice by magnetic depletion using CD43 microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany). B cells were then stained with antibodies against CD19 (clone 1D3), CD21 (clone 7G6) and CD23 (clone B3B4) (eBioscience, Frankfurt, Germany) and sorted for $CD19^+$ $CD21^+$ $CD23^+$ (FO B cells) *vs* $CD19^+$ $CD21^+$ $CD23^-$ (MZ B cells) on an ARIA II cell sorter (BD Biosciences, Heidelberg, Germany). 6×10^5 FO or 4×10^5 MZ B cells were adoptively transferred into C57BL/6 mice followed by immunization with NIP-Ficoll and application of anti-CD83 or control Ig as described in the following.

Immunization of mice

C57BL/6, BALB/c and IL-4R$\alpha^{-/-}$ mice were immunized intraperitoneally (i.p.) with 200 μg Ficoll-conjugated 4-hydroxy-3-iodo-5-nitrophenylacetyl (NIP-Ficoll; Biosearch Technologies/BioCat, Heidelberg, Germany) in 200 μl PBS. C57BL/6 or JHT mice that had received B1-8i B cells were immunized the day after adoptive transfer by i.p. injection of either 200 μg NIP-Ficoll or Ficoll-conjugated 4-hydroxy-3-nitrophenylacetyl (NP-Ficoll; Biosearch Technologies/BioCat, Heidelberg, Germany) in 200 μl PBS. For the analysis of CD83 expression kinetics *in vivo*, mice were injected i.v. with 100 μg NP-Ficoll. 20 μg rat anti-mouse CD83 mAb (clone Michel-19, Cramer et al., 2000; Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany) or control rat IgG (ChromPure rat IgG, whole molecule, Jackson ImmunoResearch Laboratories, Suffolk, UK) were injected i.p. either one day prior to Ag-challenge or on the day of cell transfer as well as one day after Ag-challenge.

Detection of serum Ig by ELISA (enzyme-linked immunosorbent assay)

Analysis of serum Igs was performed as described (Kretschmer et al., 2009). Immunized mice were bled at indicated times. After coagulation (1 h at room temperature (RT)) samples were centrifuged for 10 min at 12.000 × g. Harvested serum was stored at −20 °C for further analysis. ELISA plates were coated with 1 μg/ml NIP-BSA (Biosearch Technologies/BioCat, Heidelberg, Germany) in PBS/0.1% BSA (Albumin Bovine fraction V, Serva Elektrophoresis GmbH, Heidelberg, Germany; 50 μl/well) for the detection of NIP-specific IgM, IgG1, IgG2a and IgA. Plates were washed, blocked

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