Expression of the B7/BB1 Activation Antigen and its Ligand CD28 in T-Cell-Mediated Skin Diseases

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Interactions of CD28 (on T cells) with its recently identified ligand B7/BB1 (on antigen-presenting cells) have been shown to activate T cells via a major histocompatibility complex/Ag-independent "alternative" pathway, leading to an amplification of T-cell-mediated immune responses. The in vivo relevance of these molecules for cutaneous immunity is presently unknown. These findings prompted us to study the expression of B7/BB1 and CD28 in normal human skin and in selected T-cell-mediated inflammatory skin diseases. Biopsies were obtained from lesional skin of patients with allergic contact dermatitis, lichen planus, and, as control, from basal cell carcinoma and from healthy controls. Serial cryostat sections were stained with a panel of MoAbs directed against CD28, B7/BB1, CD3, CD1a, and KiM8 using immunohistochemistry (ABC technique). CD28 expression was observed in the majority of dermal and epidermal CD3$^+$ T cells in contact dermatitis and lichen planus. In normal skin and basal cell carcinoma, CD28 was expressed only occasionally by perivascular T cells. In allergic contact dermatitis and lichen planus, B7/BB1-expression was found on dermal dendritic cells, on dermal macrophages, on Langerhans cells, focally on keratinocytes, and occasionally on dermal T cells. No B7/BB1 immunoreactivity was detected in normal skin and basal cell carcinoma. These findings indicate that T-cell-mediated skin diseases are accompanied by an influx of CD28$^+$ T cells and an upregulation of B7/BB1 on cutaneous antigen-presenting cells, keratinocytes, and on some T cells. We speculate that "alternative" T cell-activation via the B7/CD28 pathway may contribute to the pathogenesis of these skin diseases. *Key words: B7/BB1/CD28. J Invest Dermatol 103:539–543, 1994*

R ecently, the interaction of the B7/BB1 Ag (on antigen-presenting cells) with its ligand CD28 (on T cells) has been shown to deliver activation signals to T cells distinct from those transduced via the T-cell receptor [1,2]. The B7/BB1 Ag was initially identified by two different antibodies (BB1 and B7) which were thought to recognize identical (or highly related) glycoproteins with a molecular weight of 44–54 kD. B7/BB1 shows low constitutive expression on resting B cells, monocytes, and dendritic cells; however, upon stimulation, it can be readily upregulated [3–9]. In addition, B7/BB1 is expressed on different B-cell lines, B-cell neoplasms, and on long-term activated T cells [3–5,10,11]. CD28 is a 90-kD homodimeric glycoprotein expressed by T cells, thymocytes, and plasma cells [2,12]. Binding of B7/BB1 to CD28 leads to an augmentation of several T-cell functions such as proliferation, cytokine production, adhesion, and cytotoxicity [13–17]. Defective signaling via the B7/BB1/CD28 pathway has been shown to cause T-cell tolerance [18,19].

Taken together, these studies indicate that interaction of B7/BB1 with CD28 delivers essential costimulatory signals to T cells, resulting in effective induction and amplification of T-cell-mediated immunity [1,2]. Whether B7/BB1/CD28-mediated signals play a functional role during cutaneous immune responses is presently unknown. To address this issue, we examined the expression of B7/BB1 and CD28 in T-cell-mediated skin diseases.

MATERIALS AND METHODS

Patients Patients with allergic contact dermatitis (ACD, n = 11), lichen planus (LP = 8), basal cell carcinoma (BCC, n = 12) and acute urticaria (n = 3) were compared to normal control subjects (NS, n = 9). None of the patients had received prior treatment for their skin condition. Following informed consent, full-thickness 4-mm punch biopsies were obtained from the lesional skin of the patients. In the NS group, 4-mm punch biopsies were taken from the volar aspects of the forearm. Samples were snap-frozen immediately and stored at −80°C until use.

Monoclonal Antibodies (MoAbs) MoAbs against the following human antigens were used in this study: anti-CD1a (OKT6, m-immunoglobulin[Ig]G1 Ortho Diagnostics, Inc., Raritan, NJ), anti-CD3 (IOT3, mIgG1 Biozol, München, Germany), KiM8 (mIgG1, Dianova, Hamburg, Germany), three different anti-CD28 reagents (9.3, mIgG2a, Dr. P. S. Linsley, Seattle, WA; Leu 28, mIgG1, Becton Dickinson, Sunnyvale, CA; and CLB-28/1, mIgG1, gift of Dr. Van Lier, Amsterdam, Netherlands) and three different anti-B7/BB1 reagents (BB1, mIgM, Dr. P. S. Linsley; 104, mIgG1, gift of Dr. J. Banchereau, Dardilly, France; and BB1-(IgG), mIgG1, Becton Dickinson).

Immunohistochemistry Frozen skin specimens were embedded in Optimum Cutting Medium (OCT, Miles Inc., Elkhart, IN) and 5-μm serial

cryostat sections were prepared using a Crycut 2000 (Reichert & Jung, Nußbach, Germany). Air-dried, acetone-fixed frozen sections were stained using a four-step immunohistochemical staining protocol (ABC-technique, DAKO): 1) primary MoAb (mouse IgG, IgM); 2) biotin-conjugated goat-anti-mouse IgG, or biotin-conjugated goat-anti-mouse μ-chain; 3) peroxidase-conjugated streptavidin; 4) diaminobenzidine as chromogenic substrate. Finally, sections were counterstained with hemalum. Double staining was performed as follows: 1) primary MoAb (mouse IgG, IgM); 2) biotin-conjugated goat-anti-mouse IgG, or biotin-conjugated goat-anti-mouse μ-chain (no cross-reactivity to mouse IgG); 3) peroxidase-conjugated streptavidin; 4) goat-anti-horseradish peroxidase conjugated to colloidal gold (4 nm); 5) primary MoAb (mouse IgG); 6) rabbit-anti-mouse Ig; 7) APAAP (alkaline-phosphatase and mouse anti-alkaline-phosphatase-IgG); 8) naphthol-phosphate and fast red; 9) silver enhancement (all reagents with the exception of the primary MoAb from DAKO). Control staining was performed by replacing the primary MoAb with isotype-matched control reagents. Staining was evaluated by four independent observers in a blinded fashion using a Zeiss Axioskop equipped with a MC100 camera system. The opinions of the observers were concordant. Langerhans cell enumeration was performed microscopically using an optical grid, as described [20].

RESULTS

CD28 was expressed by infiltrating lymphoid cells in ACD and LP (**Fig 1a, Table I**). Doublestaining using anti-CD28 and anti-CD3 MoAb confirmed that the majority of these CD28$^+$ cells were CD3$^+$ T cells (**Fig 1b**). CD28 was expressed by all CD4$^+$ T cells and by the majority of CD8$^+$ T cells (not shown). Staining of the same specimen with isotype-matched control MoAb excluded the possibility that this CD28 staining was nonspecific (**Fig 1c**). In normal skin, little CD28 immunoreactivity could be detected (**Fig 1d**), with the rare exception of perivascular CD28$^+$ T cells (not shown).

In allergic contact dermatitis (ACD) and LP, B7/BB1 was expressed by dendritic-shaped cells in the epidermis and dermis (**Fig 1e**). To test whether some of these dendritic cells were Langerhans cells, double staining with MoAb against CD1a and B7/BB1 was performed. These studies revealed that a variable portion of dermal and epidermal CD1a$^+$ Langerhans cells also expressed B7/BB1 (**Fig 1f,g, Table II**). In epidermis, 7%–53% of Langerhans cells were B7/BB1 positive, whereas by contrast, the number of B7$^+$/BB1$^+$ dermal Langerhans cells was significantly higher (28%–68%, **Table II**). The percentage of B7$^+$/BB1$^+$ Langerhans cells showed considerable variation depending on the disease investigated and the anti-B7/BB1 MoAb used (**Table II**). Specifically, more Langerhans cells were found to be B7$^+$/BB1$^+$ in LP than in ACD, and MoAb 104 labeled more Langerhans cells than MoAb BB1(IgG) (**Table II**). In addition, B7/BB1 was expressed by dermal macrophages of the phagocytic subtype (KiM8$^+$) and by dermal dendritic cells (**Fig 1e, Table I**).

Often, B7/BB1-positive cells of dendritic shape (i.e., Langerhans cells, dermal macrophages, or dermal dendritic cells) were found in close apposition to CD28$^+$ T cells (**Fig 1j**). This was observed more frequently in the dermis than in the epidermis. Occasionally, dermal and epidermal T cells were found to express B7/BB1, particularly when MoAb BB1(IgG) was used (**Fig 1e, Table I**).

In addition, focal B7/BB1 expression was observed on keratinocytes (**Fig 1h**). This was most pronounced in areas overlying dense dermal lymphocytic infiltrates (**Fig 1h**) and was detected best when MoAb 104 or BB1 (IgM) were used (**Table I**). Double staining demonstrated close contact of CD28$^+$ T cells and B7$^+$/BB1$^+$ keratinocytes (**Fig 1i**). Again, staining with isotype-matched control MoAb excluded the possibility that B7/BB1 staining was nonspecific (**Fig 1c**). Finally, no B7/BB1 expression could be detected in normal skin (**Fig 1k**) or in non–T-cell–mediated skin diseases such as BCC (**Fig 1l**).

DISCUSSION

This study examined the expression of B7/BB1 and its ligand CD28 in two important T-cell–mediated inflammatory skin diseases, ACD and LP. We have shown CD28 to be expressed by the majority of T cells that infiltrate the skin in ACD and LP, whereas

in normal skin, CD28 was found only occasionally on perivascular T cells.

Thus far, only few studies have examined the *in vivo* distribution of CD28, showing it to be restricted to lymphoid cells in lymph node, spleen, tonsil and thymus [21,22]. More information on the cellular distribution of CD28 has been gained from *in vitro* studies. For example, CD28 is expressed by the majority of CD4$^+$ and on 50% of CD8$^+$ T cells (particularly CTL) isolated from human peripheral blood [2,12]. In our study, all CD4$^+$ T cells infiltrating the skin expressed CD28, and approximately 70% of CD8$^+$ cells were CD28 positive.

We observed B7/BB1 to be expressed by Langerhans cells in LP and ACD but not in normal skin. Importantly, B7/BB1 was not expressed by all Langerhans cells: 1) the percentage of B7/BB1-positive Langerhans cells was higher in dermis than in epidermis; 2) more Langerhans cells were B7/BB1$^+$ in LP than in ACD; and 3) the percentage of B7/BB1-positive Langerhans cells differed depending on the anti-B7/BB1 MoAb used, raising the possibility that these reagents detect disparate forms of B7/BB1 ([23–25], detailed discussion below).

Taken together, these data suggest that Langerhans cells only express B7/BB1 upon *in vivo* activation by antigen such as contact sensitizers. Following such activation, Langerhans cells are thought to migrate from epidermis into dermis, where they enter afferent lymphatics to travel into the draining lymph nodes [26,27]. During this process Langerhans cells undergo distinct phenotypic and functional changes that have been termed "maturation" [26,27]. Specifically, Langerhans cells upregulate their surface expression of major histocompatability complex Ag, and of accessory molecules, and, as a result, increase their capacity to stimulate resting T cells [26,27]. Similar changes have been observed during short-term tissue culture of Langerhans cells [26,27]. Our hypothesis that only activated Langerhans cells express B7/BB1 is supported by recent *in vitro* studies demonstrating B7/BB1 to be expressed exclusively by cultured Langerhans cells but not by freshly isolated Langerhans cells [9,28]. Upregulation of B7/BB1 may be responsible for the "functional maturation" of Langerhans cells as they leave the epidermis. This notion is supported by our finding that close apposition of B7/BB1-positive Langerhans cells and CD28-positive T cells was more frequent in dermis than in epidermis.

Furthermore, we found B7/BB1 on dermal macrophages and dendritic cells and on some T cells in LP and ACD, but not in normal skin. This observation is consistent with *in vitro* data, reporting B7/BB1 to be expressed by activated dendritic cells, macrophages, and T cells [5,7,8,10,11,16].

In ACD and in LP we also detected focal B7/BB1 staining on keratinocytes, particularly in areas overlying dense lymphocytic infiltrates. The cellular distribution of B7/BB1 on keratinocytes resembled the "chicken-wire pattern" reported for intercellular adhesion molecule-1 (ICAM-1) [29,30]. Similar B7/BB1 staining on keratinocytes was recently detected in lesional psoriatic [31] and eczematous‡ skin. *In vitro* studies from our laboratory [32], as well as from others [31,33] indicate that activated keratinocytes express a "B7-like molecule" that differs from B7/BB1 expressed by activated B cells. Recently, CD28 ligands different from B7/BB1 have been identified and named B70, B7-2, and B7-3 [23–25]. Whether the "B7-like molecule" on KC represents B70, B7-2, or B7-3 is currently unknown. We do know however, that the "B7-like molecules" on keratinocytes, as well as B7-1 transfected into keratinocytes, serve a functional role in the binding and activation of CD28$^+$ T cells [32,34,35].

Finally, in our study, B7/BB1 expression was detected exclusively in T-cell–mediated skin diseases, but not in non–T-cell–mediated conditions or in normal skin. These findings suggest that B7/BB1 might be induced by factors released from infiltrating

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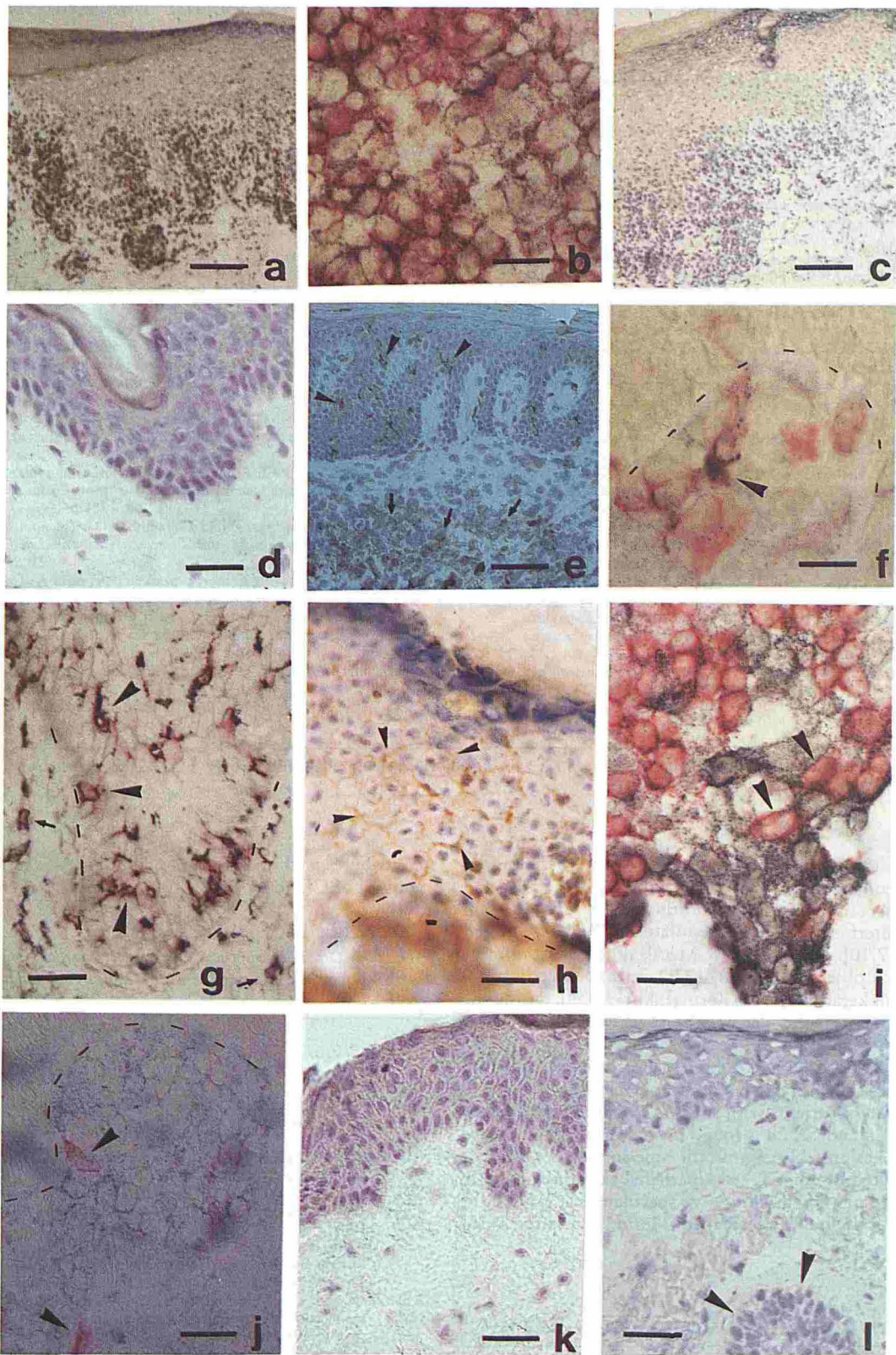
Figure 1. Expression of B7/BB1 and CD28 in T-cell–mediated inflammatory skin diseases. *a)* Lichen planus: CD28 is expressed by infiltrating T cells (MoAb Leu28; *bar, 400 μm*); *b)* allergic contact dermatitis: CD3 *(red)*, CD28 *(black)*, the majority of dermal T cells are CD3⁺/CD28⁺ (MoAbs IOT3/Leu28; *bar, 63 μm*); *c)* lichen planus: same specimen as shown in *(a)* and *(h)* stained with an irrelevant IgG1 excluding unspecific staining (X63Ag8; *bar, 400 μm*); *d)* normal skin: no CD28 immunoreactivity (MoAb Leu28; *bar, 100μm*); *e)* allergic contact dermatitis: B7/BB1 on epidermal Langerhans cells *(arrowheads)* and in dermis on dendritic cells, macrophages, and some T cells *(arrows)* (MoAb BB1-IgG; *bar, 400 μm*); *f)* allergic contact dermatitis: CD1a *(red)*, B7/BB1 *(black)*, a dermal LC is CD1a⁺/B7/BB1⁺ *(arrowhead)*, (−), dermoepidermal junction (MoAbs OKT6/104; *bar, 63 μm*); *g)* lichen planus: CD1a *(red)*, B7/BB1 *(black)*, epidermal (arrowheads), and dermal LC *(arrows)* are CD1a⁺/B7/BB1⁺, (−), dermoepidermal junction (MoAbs OKT6/104; *bar, 100 μm*); *h)* lichen planus: B7/BB1 expression by keratinocytes *(arrowheads)*, (−) dermoepidermal junction (MoAb 104; *bar, 63 μm*); *i)* allergic contact dermatitis, bullous patch test reaction: CD28 *(red)*, B7/BB1 *(black)*, close contact of CD28⁺ T cells and B7/BB1⁺ keratinocytes *(arrowheads)*, (MoAbs Leu28/104; *bar, 63 μm*); *j)* allergic contact dermatitis: CD28 *(black)*, B7/BB1 *(red)*, close contact of CD28⁺ T cells and B7/BB1⁺ dermal dendritic cells *(arrowheads)*, (−) dermoepidermal junction (MoAb Leu 28/BB1-IgG; *bar, 63 μm*); *k)* normal skin: no B7/BB1 immunoreactivity (MoAb 104; *bar, 100 μm*); *i)* basal cell carcinoma: no B7/BB1 immunoreactivity in epidermis, dermis, or tumor *(arrowhead)* (MoAb 104; *bar, 100 μm*).

Table I. Summary of the Immunohistochemical Results[a]

Disease		Epidermis			Dermis		
Ag	MoAb	Langerhans Cells	Keratinocytes	T Cells	Langerhans Cells	Dendritic Cells/Ma	T Cells
LP (n = 8)							
B7/BB1	104	++	+	(+)[b]	++	++	+[b]
	BB1(IgM)	+	+	−	+	+	−
	BB1(IgG)	+	(+)[b]	+	+	+	+[b]
CD28	Leu 28	−	−	+	−	−	+
	9.3	−	−	+	−	−	+
	CLB-28/1	−	−	+	−	−	+
ACD (n = 11)							
B7/BB1	104	++	+	(+)[b]	++	++	+[b]
	BB1(IgM)	+	+	−	+	+	−
	BB1(IgG)	+	(+)[b]	+	+	+	+[b]
CD28	Leu 28	−	−	+	−	−	+
	9.3	−	−	+	−	−	+
	CLB-28/1	−	−	+	−	−	+
BCC (n = 12)							
B7/BB1	104	−	−	−	−	−	−
	BB1(IgM)	−	−	−	−	−	−
	BB1(IgG)	−	−	−	−	−	−
CD28	Leu 28	−	−	−	−	−	(+)[b]
	9.3	−	−	−	−	−	−
	CLB-28/1	−	−	−	−	−	(+)[b]
NS (n = 9)							
B7/BB1	104	−	−	−	−	−	−
	BB1(IgM)	−	−	−	−	−	−
	BB1(IgG)	−	−	−	−	−	−
CD28	Leu 28	−	−	−	−	−	(+)[b]
	9.3	−	−	−	−	−	−
	CLB-28/1	−	−	−	−	−	(+)[b]

[a] Staining was evaluated in a semiquantitative fashion as described [20]: ++, strong specific staining; +, specific staining; (+), weak specific staining; −, no immunoreactivity.
[b] A minority of cells stain positively with the MoAb.

$CD28^+$ T cells. This hypothesis is supported by 1) our own finding of close physical contact of $CD28^+$ T cells and $B7^+/BB1^+$ cells, and 2) the *in vitro* observation that T-cell products such as interleukin (IL)2, IL4, and interferon (IFN)γ upregulate B7/BB1 on antigen presenting cells [7,10]. Using anti-B7 MoAb different from ours, other investigators observed faint B7/BB1 immunoreactivity on Langerhans cells or keratinocytes in normal skin [33,34], raising the possibility that their reagents detect other CD28-ligands such as B7-2, B7-3, or B70 [23–25].

We conclude that B7/BB1 is expressed by professional cutaneous antigen-presenting cells, focally by keratinocytes, and by some T cells in lesional skin of patients with allergic contact dermatitis and lichen planus. Its ligand CD28 is found on the majority of T cells infiltrating these lesions. No significant amounts of B7/BB1 or CD28 were detected in non–T-cell–mediated skin diseases or in normal skin. Based on these findings, we speculate that B7/BB1 is induced during T-cell–mediated cutaneous immune responses, and

contributes within skin to the adhesion, activation, and cytotoxic activity of $CD28^+$ T cells.

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Table II. B7/BB1-Expression by Langerhans Cells[a]

Disease	% of B7/BB1$^+$/CD1a$^+$ Langerhans Cells (± SD)	
MoAb	Epidermis	Dermis
LP		
104	53 ± 14.7	68 ± 4
BB1(IgG)	7 ± 2.6	41 ± 11.5
ACD		
104	24 ± 8.7	55 ± 19
BB1(IgG)	10 ± 0.7	28 ± 14

[a] Langherhans Cell enumerations were performed as described [20]. Pooled data from patients with LP (n = 5) and ACD (n = 7) are expressed as mean % of CD1a$^+$/ B7$^+$ cells ± SD.

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