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*J Immunol* 2001; 167:132-139; doi: 10.4049/jimmunol.167.1.132

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Enhancement of CD8\(^+\) T Cell Responses by ICOS/B7h Costimulation

Jeffrey J. Wallin, Linda Liang, Anastasia Bakardjieva, and William C. Sha

Although the recently identified ICOS/B7h costimulatory counterreceptors are critical regulators of CD4\(^+\) T cell responses, their ability to regulate CD8\(^+\) responses is unclear. Here we report using a tumor-rejection model that ectopic B7h expression can costimulate rejection by CD8\(^+\) T cells in the absence of CD4\(^+\) T cells. Although responses of naive T cells were significantly augmented by priming with B7h, B7h was surprisingly effective in mobilizing recall responses of adoptively transferred T cells. To explore why secondary responses of CD8\(^+\) T cells were particularly enhanced by B7h, kinetics of ICOS up-regulation, proliferative responses, and cytokine production were compared from both naive and rechallenged 2C-transgenic T cells costimulated in vitro. Although B7h costimulated proliferative responses from both CD8\(^+\) populations, rechallenged cells were preferentially costimulated for IL-2 and IFN-\(\gamma\) production. These results indicate that ICOS/B7h counterreceptors likely function in vivo to enhance secondary responses by CD8\(^+\) T cells. The Journal of Immunology, 2001, 167: 132–139.

T cell recognition of Ag in association with MHC (signal 1) is a necessary prerequisite for the initiation of T cell activation. However, complete activation of naive T cells requires a second Ag-independent signal through costimulatory receptors. Signal 2 is provided by CD28 interacting with one of its ligands (B7.1 or B7.2) on professional APC. Costimulation provided by the constitutively expressed CD28 is responsible for inducing high-level IL-2 production and survival of naive T cells (1–3). Interactions of either B7.1 or B7.2 with CTLA-4, a homolog of CD28 expressed on T cells, results in inhibition of T cell responses (4, 5).

Recently, a homolog of CD28 was identified in a screen for activation Ags on T cells (6). This molecule, ICOS, enhances proliferation of T cells as well as the production of several cytokines (IL-4, IL-5, GM-CSF, TNF-\(\alpha\), IFN-\(\gamma\), and IL-10). ICOS is expressed only on activated T cells and does not interact with B7.1 or B7.2. Instead it binds to B7h (B7RP-1, GL-50, ICOSL), a recently identified molecule with homology to B7.1 and B7.2 (7–10). B7h is expressed on B cells, macrophages, and dendritic cells and has been shown to provide costimulation in vitro and enhance B cell/T cell proliferation and differentiation in vivo (7–9, 11). In contrast to CD28 costimulation, ICOS may be providing costimulatory signals to previously activated T cells based on its inducible expression shortly after T cell activation.

ICOS is expressed on both CD4\(^+\) and CD8\(^+\) T cells and appears to enhance T cell-dependent Ab responses and cytokine production from CD4\(^+\) cells (6, 8, 12). However, the potential function of ICOS expressed on CD8\(^+\) T cells in the generation of MHC class I-specific CTL is less well understood. Activation of effective CD8\(^+\) responses does not always require exogenous help from CD4\(^+\) Th cells, which may only be required when TCR affinity is low (13, 14). CD8\(^+\) T cells can be stimulated to proliferate and generate CTL responses to alloantigens in the absence of CD4\(^+\) Th cells (15–17). It has been demonstrated that costimulation of naive CD8\(^+\) T cells through CD28 allows for the induction of CTL responses without exogenous help (18).

We previously reported that transcription of the B7h gene could be induced in 3T3 and embryonic fibroblast cell lines treated with TNF-\(\alpha\) and that B7h was basally expressed in several nonlymphoid tissues of healthy mice (7). To explore the potential in vivo significance of these observations, we used a murine tumor model to examine the consequences of B7h expression on cells in nonlymphoid tissues. Ectopic expression of B7h resulted in enhanced tumor rejection that could be mediated by CD8\(^+\) T cells in the absence of CD4\(^+\) T cells. Although B7h was able to augment anti-tumor responses by both priming responses of naive T cells and by augmenting secondary recall responses of CD8\(^+\) T cells, the enhancement of recall responses by B7h was striking. To examine why B7h was particularly effective in enhancing recall responses, we examined different effector responses of CD8\(^+\) T cells in vitro using transgenic T cells, where the antigenic specificity of responder cells is well defined. We observed an in vitro correlate in the ability of B7h to costimulate cytokine production from rechallenged CD8\(^+\) T cells but not naive CD8\(^+\) T cells. These data suggest that ICOS/B7h interactions may play a significant role in regulating secondary cytotoxic T cell responses.

Materials and Methods

Mice

Six-week-old female BALB/c nude mice and A/J mice (H-2K\(^b\), I-A\(^b\), I-E\(^b\), and D\(^b\)) were obtained from Simonsen (Gilroy, CA) and The Jackson Laboratory (Bar Harbor, ME), respectively. 2C TCR-transgenic mice in the C57BL/6 background were obtained from a colony maintained at our institution. All mice were used within 2 mo of receipt in accordance with the animal use guidelines of our institution.

Cells

P815 mastocytoma cells (H-2\(^d\), MHC class II\(^d\) ) were cultured in RPMI 1640 containing complete medium (10% FCS, 25 \(\mu\)M 2\(\beta\)-ME, 2 mM l-glutamine, 100 U/ml penicillin, and 100 \(\mu\)g/ml streptomycin). The chemically induced fibrosarcoma cell line Sa1N (H-2K\(^d\), I-A\(^b\), I-E\(^b\), D\(^b\)) (19) and the human kidney cell lines 293T and BOSC23 were cultured in DMEM supplemented with complete medium.
cDNAs and plasmids

The B7h-Ig version used in this paper is modified from our original paper (7). A myc-tagged version was created by subcloning a KpnI-BamHI B7h insert from our original B7h-Ig and a BamHI-XhoI insert containing the CH2-CH3 domains of mouse IgG1 that was generated by PCR using the following oligonucleotides: 5'-GGCAATCAGCCCGGTGGTTGTTGCT-3' and 5'-GGCAATCAGCCCGGTGGTTGTTGCT-3'. Both inserts were ligated in-frame in a trigonal sequence into the pcDNA4-myc-his vector (Invitrogen, San Diego, CA) and transfected into 293T cells for protein expression.

Generation of Sa1N and P815 clones

Because Sa1N cells are highly immunogenic, introduction of green fluorescence protein (GFP)3 as a marker did not alter the growth kinetics of GFP-expressing tumors in comparison to wild-type tumors in syngeneic mice. We observed no difference in growth of the four control Sa1N clones expressing GFP over wild-type Sa1N tumors in A/J and nude mice (data not shown). Consequently, all Sa1N and P815 tumors clones were generated using a GFP marker to facilitate cloning. A B7h-GFP protein, previously characterized as functionally equivalent to wild-type B7h, was generated by PCR cloning the B7h cDNA into the pEGFP-N3 mammalian expression vector (Clontech Laboratories, Palo Alto, CA) and then subcloned into the Sall-NorI sites of a murine stem cell virus (MSCV) retroviral vector. A murine B7.2 cDNA was cloned into a MSCV-internal ribosomal entry sequence (IRES)-GFP retroviral vector using an IRES GFP marker (20). Control clones were generated in parallel using cells transduced with empty MSCV-IRES-GFP retroviral vector. Flow cytometric analysis of GFP expression was equivalent (within a 3-fold range of mean fluorescence over background) in all Sa1N clones tested, consistent with the comparable staining observed with an anti-B7h mAb (22D7) for the B7h-Sa1N clones (see Fig. 1A) and with an anti-B7.2 mAb (clone GL1; PharMingen, San Diego, CA; data not shown) for the B7.2-Sa1N clone used in Fig. 2. Flow cytometric analysis of GFP expression on B7h- and B7.2-transduced P815 clones was also within a 3-fold range of mean fluorescence over background. High-titer helper-free retroviral stocks were produced as previously described (7). For the generation of clones, 24 h following infection cells were aliquoted into 96-well plates at 0.5 cell/well. GFP-positive clones were visible by fluorescence microscopy after 2–3 wk of culture. At that time, cells from GFP-positive wells were replated at a concentration of 0.5 cell/well, and positives were expanded and tested for expression.

Tumor inoculation and growth

All primary tumor inoculations were performed by injecting 5 × 10^5 cells s.c. into the left flank of A/J or BALB/c nude mice. Nernier calipers were used to measure the tumors in two dimensions according to previously established protocols (21, 22). A minimum of four mice was used for each experimental group, and tumor growth was monitored in individually identified animals. All contralateral or secondary injections were made by injecting 5 × 10^5 cells s.c. into the right flank. For all i.p. immunizations, 2 × 10^6 irradiated cells were used.

Antibodies

A mAb to B7h (22D7) was generated by immunization of Syrian hamsters with Chinese hamster ovary cells expressing B7h. For flow cytometric analysis (Fig. 1A) an anti-hamster IgG PE Ab (Caltag, South San Francisco, CA) was used as a second-step reagent. Hybridomas GK1.5 (anti-CD4) and 2.43 (anti-CD8) were obtained from American Type Culture Collection (Manassas, VA) and were maintained in RPMI 1640 supplemented with complete medium. GK1.5 and 2.43 cells were used to generate ascites in BALB/c nude mice. Ab production was verified by the use of ascites in flow cytometry and quantified by Western blot using an anti-Rat HRP Ab (Caltag) for detection and a rat IgG Ab (Sigma, St. Louis, MO) as a standard. An anti-myc Ab (9E10) was obtained from Santa Cruz Biotechnology (Santa Cruz, CA) and was used in flow cytometry to detect positive B7h-Ig binding.

In Vivo T cell depletion

CD4+ and CD8+ T cells were depleted from A/J mice by i.p. injection of ascites equivalent to 0.1 mg GK1.5 or 0.2 mg 2.43, respectively, on three instances 1 wk before s.c. injection of tumor. Control mice were injected with the same dose of purified rat IgG (Sigma). Throughout the experiment, two weekly injections of the same dose were administered i.p. to maintain the depletions. Before the tumor experiment was initiated, one spleen was harvested from each group, and depletion of the relevant subset was verified by flow cytometry with a separate anti-CD4 Ab or anti-CD8 Ab, which are not blocked by the depleting GK1.5 and 2.43 Abs. All mice tested in these studies exhibited >98% depletion of the appropriate T cell subset. For transfer experiments, naive mice were depleted of CD4+ T cells by three injections of the GK1.5 Ab before immunization with 2 × 10^6 irradiated Sa1N tumor cells. Four injections of the GK1.5 Ab were given in the 2 wk following immunization. Animals were sacrificed, and lymph node T cells were isolated, purified, and transferred. The percentage of transferred CD8+ T cells was >96%.

Cell proliferation and cytokine assays

Lymph node or in vitro expanded CD8+ T cells were purified as previously described with the inclusion of GK1.5 (anti-CD4) to the complement reagent (7). Final percentages of CD8+ T cells for primary and rechallenge experiments were 97 and 99%, respectively. Purified cells were labeled with CFSE (Molecular Probes, Eugene, OR) according to the supplier’s protocol. Following CFSE labeling, CD8+ T cells (8 × 10^5) were incubated with irradiated control, B7h, or B7.2 clones (4 × 10^5) in flat-bottomed plates (24-well) and analyzed by flow cytometry on day 5 (rechallenge) or day 7 (primary). Supernatants from the in vitro proliferation experiments were collected at 24 h of culture and assayed for the presence of cytokines by sandwich ELISA. Naive T cells were checked for absence of expression activation markers including CD69 and CD25. For detection of IL-10 in supernatants we used an IL-10 ELISA kit from PharMingen. Abs for the IL-4 and IFN-γ ELISAs were gifts from K. M. Murphy and R. D. Schreiber (Washington University Medical School, St. Louis, MO). Abs for the IL-2 ELISAs were gifts from J. P. Allison (University of California, Berkeley, CA). All samples, including controls, were assayed in triplicate.

Generation of cytotoxic T cells and cytolytic assays

Responder splenocytes from 2C TCR transgenic mice were cocultured with irradiated stimulator BALB/c splenocytes in complete medium at 37°C for 5 days at a 2:1 stimulator-responder ratio. 31Cr-labeled P815 target clones were incubated with the expanded 2C T cells in round-bottom 96-well plates at different ratios. 31Cr-containing supernatants were counted after 6 h. All samples, including controls, were assayed in triplicate.

Results

B7h enhances anti-tumor responses of CD8+ T cells

To investigate the potential role of B7h expression observed in nonlymphoid tissues, we used a well-established murine tumor model that allowed us to quantitate the immune response elicited by B7h by measuring tumor growth. The murine fibrosarcoma cell line Sa1N expresses MHC class I, but does not express MHC class II, and is negative for B7.1, B7.2, and B7h expression (Ref. 19, data not shown). Sa1N is a highly immunogenic tumor that stimulates an ineffective, but specific, immune response in vivo (23). Because of its high degree of immunogenicity, Sa1N tumor growth is unaffected by the intracellular expression of marker proteins like GFP. In contrast, surface expression of B7.1 on Sa1N cells has been shown in several previous studies to significantly enhance anti-tumor responses resulting in rapid tumor rejection (21, 24).

Immune responses to Sa1N cells expressing B7h were evaluated by monitoring tumor growth of B7h-expressing Sa1N cells (B7h-Sa1N) in comparison to control Sa1N cells (control Sa1N). Individual clones of Sa1N cells expressing B7h and control clones were generated by retroviral transduction (Fig. 1A). Tumor growth was assessed every 2–3 days following s.c. injection into syngeneic A/J mice throughout the duration of the experiment or until tumors became ulcerated (Fig. 1B). Progressive growth of control Sa1N tumors was observed in all four clones tested. However, ectopic B7h expression resulted in rejection of Sa1N tumors in all
four clones tested. B7h-Sa1N clones initially grew equivalently to control clones for the first 11 days, but between days 11 and 13 became static in comparison to control Sa1N tumors. By day 20, most B7h-Sa1N tumors had completely regressed. Residual B7h-Sa1N tumors remaining in mice at day 20 either remained static or continued to regress as long as mice were maintained (up to 3 mo). The ability of B7h to augment anti-tumor responses was dependent upon host T cell responses, as all of the clones grew progressively in nude mice. Equivalent growth in nude mice was observed for the B7h-Sa1N (number 4) and control Sa1N (number 4) clones used in subsequent experiments (Fig. 1C).

To determine which subset(s) of T cells were involved in B7h enhancement of immune responses to tumors, A/J mice were depleted of either CD4⁺ or CD8⁺ T cells before inoculation with B7h-Sa1N cells (Fig. 1D). Effectiveness of depletions with specific and control Abs was confirmed at the initiation and twice during the experiment by flow cytometric analysis. In each case, >98% depletion of the respective T cell subset was observed (data not shown).
shown). A/J mice depleted of CD8+ T cells were unable to respond to B7h-Sa1N cells, and tumors grew progressively for the entirety of the experiment. In contrast, mice depleted of CD4+ T cells eliminated B7h-Sa1N tumors with only slightly delayed kinetics in comparison to mice treated with a control Ab. These data indicate that CD8+ T cells were capable of mediating rejection of B7h-Sa1N tumors in the absence of CD4+ T cells.

B7h enhances priming by live tumor cells of responses of naive T cells

We next sought to address whether B7h was effective in costimulating tumor rejection by acting early in the response to enhance priming of naive T cells or by acting later in the response to enhance responses of T cells that had been primed. Emerging data for regulation of CD4+ T cell responses suggest that ICOS/B7h interactions play a more significant role in regulating secondary immune responses than in primary responses where CD28 interactions with B7.1 and B7.2 appear more critical (12). Because rejection of B7h-Sa1N tumors could be mediated entirely by CD8+ T cells, we used the Sa1N tumor model to examine whether an analogous difference existed in the ability of B7h to regulate primary and recall responses of CD8+ T cells.

To assess the effectiveness of B7h early in the response, different s.c. tumors were allowed to grow for 3 days before a second control tumor was introduced at a contralateral site (Fig. 2A). Growth of this second tumor was measured and used to assess the effectiveness of priming by B7h- or B7.2-expressing clones in comparison to priming by control clones or no priming. Under these conditions, control clones were only modestly effective at priming responses. In contrast, s.c. priming with a B7.2-Sa1N clone resulted in enhanced rejection of control Sa1N tumors. Subcutaneous priming with B7h-Sa1N clones also inhibited growth of control Sa1N tumors, but somewhat less effectively than a B7.2-Sa1N clone. These differences in priming by live tumor cells were unlikely to have resulted from differences in antigenicity of clones, because all clones prevented growth of a second tumor under conditions where cross-presentation of tumor Ags by host APCs was maximized by i.p. immunization with irradiated cells (Fig. 2B). These results indicate that B7h can act early in an in vivo response to directly prime responses of naive T cells.

B7h markedly augments recall responses of CD8+ T cells

We next examined the ability of B7h to augment anti-tumor activity in secondary recall responses of T cells. Rejection of B7h-Sa1N tumors resulted in strong protective host immunity to rechallenge with Sa1N cells. Mice that had rejected B7h-Sa1N tumors and were rested for at least 4 wk showed no tumor growth upon rechallenge with either control Sa1N or B7h-Sa1N cells introduced 3 days after priming under different conditions with control Sa1N, B7h-Sa1N, or B7.2-Sa1N clones. Under conditions of maximal cross-priming, all Sa1N clones are able to prime against growth of a second tumor. Mice were immunized i.p. with irradiated control Sa1N, B7h-Sa1N, or B7.2-Sa1N cells and challenged 3 days later by s.c. injection of control Sa1N tumor cells.

Adaptive transfer of 10^7 naive T cells had no effect on growth of either tumor over the period from days 7–11 (Fig. 3A). In marked contrast, adoptive transfer of 10^6 or 10^7 T cells from immunized mice resulted in rapid and efficient rejection of B7h-Sa1N tumors, whereas little or no impairment in growth of contralateral control tumors was observed. Even at the lowest dose of 10^5 transferred T cells, B7h-Sa1N tumors were still rejected. Thus, B7h was extremely rapid and efficient in mobilizing anti-tumor responses in secondary recall responses. The magnitude of this enhancement of recall responses by B7h is highlighted by the inability of the highest dose of transferred T cells to alter growth of the contralateral control tumor.

To confirm that these rapid recall responses were mediated by CD8+ T cells, we also transferred recall responder CD8+ T cells, purified from mice depleted of CD4+ T cells that were immunized with irradiated control-Sa1N cells (Fig. 3B). Adoptive transfer of 10^7 recall responder CD8+ T cells resulted in significant growth
inhibition of both control-Sa1N and B7h-Sa1N tumors, although B7h-Sa1N tumors were still ablated with much greater efficiency. The growth inhibition of control-Sa1N tumors observed in this experiment likely reflects the increased number of cytolytic effectors in transferred CD8\(^+\) T cells that result from i.p. immunization (see Fig. 2).

These in vivo experiments suggested that B7h was effective in augmenting anti-tumor responses by CD8\(^+\) T cells in both primary and secondary recall responses, but that enhancement of recall responses by B7h was particularly effective. To examine why B7h was particularly efficacious in enhancing recall responses, we examined different responses of CD8\(^+\) T cells in vitro using transgenic T cells, where the antigenic specificity of responder T cells can be well controlled. For these studies, we used transgenic 2C TCR T cells and P815 target cells that expressed the L\(^d\) alloantigen (25). Individual clones of P815 cells were generated that were transduced with retroviral vector alone (control clones), a vector expressing B7h (B7h clones), or a vector expressing B7.2 (B7.2 clones).

B7h costimulates in vitro responses of CD8\(^+\) T cells

Because of the rapidity of secondary responses to B7h-expressing tumors, we first examined the ability of B7h to enhance lytic effector function of CD8\(^+\) T cells (Fig. 4). Expression of B7.1 or B7.2 by target cells has previously been shown to be insignificant for effector function of CTL (26). 2C T cells from MLC were assayed for lytic activity against control, B7h, or B7.2 target cells. No enhanced lysis of B7h target cells was observed at multiple E:T ratios. These results indicate that B7h expression on target cells, like B7.1 or B7.2, did not augment cytolytic activity of activated CD8\(^+\) cells.

We next examined the kinetics of up-regulation of B7h counterreceptor(s) expressed on activated CD8\(^+\) T cells (Fig. 5A). Previous studies have shown that B7h interacts with the cell-surface receptor ICOS that is not expressed on naive T cells but is induced upon T cell activation (8–10). Kinetics of surface ICOS expression and other potential B7h counterreceptors on activated CD8\(^+\) T cells were examined by flow cytometry using a B7h-Ig fusion protein that binds ICOS (Fig. 5A). In primary MLC of transgenic and

FIGURE 3. B7h markedly augments recall responses of CD8\(^+\) T cells. All measurements shown are of growth of two contralateral tumors in the same mice, a control Sa1N (right flank) and a B7h-Sa1N tumor (left flank), before and after i.v. transfer of different T cell populations. A, Two contralateral tumors grown equivalently through day 11. Adoptive transfer of naive T cells does not alter the equivalent growth of the two contralateral tumors. However, adoptive transfer of rested T cells from mice that had previously rejected Sa1N tumors results in rapid rejection of the B7h-Sa1N tumor, but not the contralateral control tumor. Transfers were on day 7 of tumor growth (arrow) and the number of purified (>98%) T cells transferred is indicated above each arrow. Error bars represent SD of measurements from five mice. B, Recall responses of CD8\(^+\) T cells sufficient to mediate rapid rejection of B7h-expressing tumors. Transferred CD8\(^+\) T cells were purified from CD4-depleted mice that had been rested 14 days after immunization with irradiated Sa1N tumor cells. Error bars represent SD of measurements from four mice.

FIGURE 4. Lytic effector function of activated CD8\(^+\) T cells is not altered by B7h expression on target cells. 2C transgenic T cells were expanded in vitro for 5 days and tested for lytic effector function against 51Cr-labeled P815 target cells transduced with either a control retroviral vector or retroviral vectors expressing B7h or B7.2, at the E:T ratios indicated.
irradiated BALB/c splenocytes, a large fraction of CD8⁺ T cells stained positively with B7h-Ig by 20 h. By 44 h, all CD8⁺ T cells were positive, and staining was maximal. Staining with B7h-Ig was maintained for at least 6 days, but by day 8 had become undetectable in resting cells. When these resting CD8⁺ T cells were rechallenged with BALB/c stimulators, the entire population of CD8⁺ T cells shifted in staining with B7h-Ig by 20 h, suggesting that up-regulation of ICOS was somewhat more rapid in rechallenged cells than in naive cells. Surprisingly, the staining observed with B7h-Ig in rechallenged CD8⁺ T cells did not reach the levels or duration seen in a primary MLC.

To examine the ability of B7h to costimulate proliferation of CD8⁺ T cells, we tested CD8⁺ transgenic T cells purified from both lymph nodes of 2C TCR mice and from 2C splenocytes expanded once in a MLC (Fig. 5B). Purified CD8⁺ T cells were labeled with CFSE and cocultured with control, B7h, or B7.2 clones. Because proliferative responses of CD8⁺ T cells from naive mice were weaker than responses of previously activated cells, cells were monitored by flow cytometric analysis after incubation with control, B7h, or B7.2 clones. The flow cytometric analyses are of day 7. For the rechallenge experiment, 2C TCR T cells were expanded in vitro for 7–8 days. CD8⁺ T cells were purified (>99%) from resting cultures and restimulated with different clones at a 1:5 ratio. The flow cytometric analyses are of day 5 following restimulation. Similar results were observed for two independent control, B7h, or B7.2 clones.
assayed by flow cytometry at day 7 for primary responses, and at day 5 for rechallenge responses. In responses of both naive and rechallenged CD8+ T cells, B7h clones enhanced proliferation of CD8+ T cells in comparison to control clones, although the enhancement of proliferation was somewhat less effective than B7.2 clones with respect to both the fraction of cells proliferating and number of cellular divisions. Thus, ectopic B7h expression costimulated in vitro proliferation of both naive and rechallenged CD8+ T cells.

Although proliferative responses were similar between naive and rechallenged cells, B7h clones costimulated differential amounts of cytokine production from naive and rechallenged CD8+ T cells (Fig. 5C). For both naive and rechallenged cells, little IL-10 or IL-4 was elicited in cultures costimulated with either B7h or B7.2 clones. In rechallenged cells stimulated by B7h clones, levels of IL-2 and IFN-γ were elicited that were comparable to the levels produced by cells stimulated with B7.2 clones. In contrast, levels of IL-2 and IFN-γ from naive cells stimulated by B7h clones were significantly less than the cytokine levels produced by cells stimulated with B7.2 clones, and, instead, were comparable to levels produced from control clones. These data suggest that B7h was more effective in costimulating IL-2 and IFN-γ production from rechallenged CD8+ T cells than from naive CD8+ T cells.

Discussion

Our results with the Sa1N tumor model demonstrate that the third member of the B7 family of costimulatory ligands, B7h, can enhance in vivo primary and secondary responses of CD8+ T cells, and in vitro enhances proliferative responses, as well as IL-2 and IFN-γ, but not IL-10 or IL-4, production, from CD8+ T cells. It is well known that help provided by CD4+ Th cells can augment a CTL response. However, sufficient effector CD8+ CTL can also be generated in the absence of CD4+ T cells (18). Rejection of B7h-expressing tumors, like B7.1-expressing tumors (27, 28), was not dependent on CD4+ T cell help. This ability of B7h to directly costimulate CD8+ T cells expressing ICOS may be critical in augmenting anti-tumor responses to Sa1N, particularly in light of recent studies indicating that ICOS/B7h interactions preferentially augment Th2 responses (12, 29). Activation of ICOS in human T cells was originally shown to costimulate production of the Th2 cytokine IL-10 involved in inhibition of Th1 responses (6). More recently, ICOS was shown to be important as a costimulatory receptor for both recently activated cells and for Th2 but not Th1 effector cells (12).

A striking feature of costimulation by ICOS/B7h is the emerging data indicating that ICOS/B7h interactions play a more significant role in regulating secondary immune responses than in primary responses where CD28-B7.1 and B7.2 interactions appear more critical. In a model of contact hypersensitivity, activation of ICOS at the time of challenge was found to be significantly more effective in exacerbating contact hypersensitivity than when ICOS was activated at the initial sensitization phase (8). Blockade of ICOS activation was also found to be more potent than blockade of CD28 activation in inhibiting cytokine production from recently activated CD4+ T cells, but not from naive CD4+ cells where CD28 blockade was more critical (12).

Our results with a tumor model suggest that ICOS/B7h interactions are also more potent in enhancing secondary recall responses of CD8+ T cells than in primary responses. In primary responses to tumors, ectopic B7h expression augmented tumor rejection, but inhibition of tumor growth was observed only after 11 days of tumor growth. However, in secondary responses to tumors, ectopic B7h expression was highly efficient and rapid in enhancing tumor rejection by CD8+ T cells. This ability of B7h to rapidly mobilize tumor rejection in secondary responses did not appear to result from enhancement of CTL lytic effector function. Because B7h was found to directly costimulate proliferation of CD8+ T cells in vitro, it is likely that direct priming and expansion of CD8+ T cells contributed to enhanced rejection of B7h-expressing tumors. Consistent with previous studies demonstrating enhanced cytokine production from rechallenged over naive T cells (30), we observed that B7h preferentially costimulated IL-2 and IFN-γ production from rechallenged CD8+ T cells over naive cells in comparison to B7.2, suggesting a potential basis for the effectiveness of B7h in enhancing in vivo secondary responses.

The ability of ICOS/B7h interactions to enhance anti-tumor responses of CD8+ T cells to Sa1N suggests that B7h may also be involved in regulation of CD8+ T cells in other contexts. However, in a model of lymphocytic choriomeningitis virus and vesicular stomatitis virus infection where ICOS regulation was examined, ICOS was shown to regulate CD4+ responses of Th1 and Th2 subsets, but not CTL responses (31). Although CTL responses were not found to be regulated by ICOS, only primary responses, and not secondary responses, were examined in these viral infection models. It will be of interest to examine the role of ICOS/B7h in memory CD8+ T cell responses, particularly given the observations that B7h is found expressed at low levels in peripheral tissues of normal mice and can in vitro be induced on fibroblast cell lines treated with the inflammatory cytokine TNF-α (7).

Acknowledgments

We thank J. Allison and J. Ziskin for advice on tumor studies, D. Raulet for 2C TCR mice and assistance with CTL assays. We thank J. Allison and members of the Sha laboratory for critical comments on the manuscript. W.C.S. is a Pew Biomedical Scholar.

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