Alloantigen-Induced Human Lymphocytes Rendered Nonresponsive by a Combination of Anti-CD80 Monoclonal Antibodies and Cyclosporin-A Suppress Mixed Lymphocyte Reaction in Vitro[1]

Patrizia Comoli,* Daniela Montagna,* Antonia Moretta,* Marco Zecca,† Franco Locatelli,† and Rita Maccario[2]*

Induction of a state of long-term, alloantigen-specific T cell nonresponsiveness has significant implications for human transplantation. It has been previously described that alloantigen-specific anergy may be induced by addition of cyclosporin-A together with anti-CD80(B7–1) mAb to a MLR. In this study we endeavored to verify whether alloantigen-induced PBL rendered anergic by the addition of a combination of anti-B7 mAb and cyclosporin-A during a MLR had a suppressive effect when added to autologous lymphocytes activated in MLR. We found that: 1) the addition of cells rendered anergic by this procedure to a MLR suppress both proliferative and cytotoxic response of autologous responsive PBL to either the same or third-party stimulator cells; 2) the suppressive effect is limited to alloantigen-induced T cell activation, as addition of anergic cells does not influence mitogen- or antigen-induced proliferation of autologous responsive T cells; 3) nonresponsiveness of suppressed cells cannot be reversed by either subsequent restimulation with allogeneic cells or addition of exogenous IL-2 to the cultures; 4) the suppressive effect is apparently not due to secretion of anergic cell-derived soluble factors, but it seems to be dependent on cell-cell contact between anergic, responsive, and stimulator cells. These data suggest that: 1) the delivery of a direct signal mediated by anergic lymphocytes through a cell-cell contact is likely to be the mechanism responsible for the suppressive effect here described; 2) anergic cells may propagate alloantigen-specific tolerance to potentially responsive autologous lymphocytes. Preliminary experiments indicate that anti-CD86(B7–2) mAb may play a similar role in the generation of alloantigen-induced nonresponsiveness. *The Journal of Immunology,* **1995, 155: 5506–5511.**

The interaction between CD28 molecule expressed on the majority of T lymphocytes and B7 family ligands expressed on APC is known to be capable of delivering an important costimulatory signal for T cells and is responsible for cyclosporin A (Cs-A)[3]-resistant T lymphocyte activation (1–5). In particular, several evidences indicate that B7/CD28 costimulatory pathway plays a relevant role during MLR (6–10). In keeping with these data, Van Gool et al. reported that the combination of anti-B7 mAb and Cs-A can synergistically block alloantigen-induced T cell activation in vitro (11, 12). The same authors showed that the nonresponsiveness thus observed resulted from the induction of a state of alloantigen-specific anergy and was not caused by clonal deletion. Recently, it has been demonstrated that antigen-specific T cell clones rendered nonresponsive by stimulation with specific ligand in the absence of any added APC could suppress autologous T cells with the same specificity, thus suggesting that anergic T lymphocytes may exert an active immunoregulatory effect on the induction of tolerance (13). These studies raised the possibility of manipulating CD28/B7 costimulatory interactions toward the induction of immunosuppression in vivo, thereby offering new strategies for the prevention of graft rejection and graft-vs-host disease in the context of solid organ and bone marrow transplantation.

In support of this hypothesis, we tested whether alloantigen-induced PBL rendered anergic by the addition of a combination of anti-CD80(B7–1) mAb and Cs-A during a MLR had a suppressive effect when added to autologous T lymphocytes activated in MLR with the same stimulators. We found that the addition to a MLR of cells rendered anergic by this procedure suppresses both proliferative and cytotoxic response of autologous responsive PBL to either the same or third-party stimulator cells. The suppressive effect seems to be dependent on cell to cell contact between anergic, responsive, and stimulator cells and is apparently limited to alloantigen-induced T cell activation, since it does not influence mitogen- or Ag-induced proliferation of autologous responsive cells.

Materials and Methods

Isolation of PBL

Heparinized peripheral blood was obtained from healthy controls or from donor/recipient pairs of HLA-matched unrelated bone marrow transplantation. PBL were isolated by Ficoll-Hypaque density gradient centrifugation, resuspended in RPMI 1640 medium (Life Technologies, Paisley, Scotland) supplemented with 2 mM L-glutamine, 50 μg/ml gentamicin, and 10% FCS (complete medium).

Monoclonal Abs

mAb employed in the functional in vitro assays and for surface marker analysis include: anti-BB1/B7 (CD80) (IgG1), anti-HLA DR FITC (IgG2a), anti-IL-2R (p55) (CD25) PE (IgG1), anti-Leu-4 FITC (CD3)

*Immunology Laboratory, and †Bone Marrow Transplant Unit, Department of Pediatrics, University of Pavia, IRCCS Policlinico San Matteo, Pavia, Italy

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[2] Address correspondence and reprint requests to Dr. Rita Maccario, Laboratory of Immunology, Department of Pediatrics, University of Pavia, IRCCS Policlinico San Matteo, p.le Golgi 2, 27100 Pavia, Italy.

[3] Abbreviations used in this paper: Cs-A, cyclosporin A; PE, phycoerythrin; TT, tetanus toxoid, PPD, purified protein derivative.

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(IgG1), anti-Leu-M3 PE (CD14) (IgG2b), (Becton Dickinson, Mountain View, CA), anti-B7–2 (CD86) (IgG1) (Ancell, Bayport, MN), and anti-IL-2-Rb (p75) FITC (IgG 1) (Endogen, Boston, MA). Phenotypic analysis of cell populations was performed by means of direct immunofluorescence on a FACScan flow cytometer (Becton Dickinson) (14).

To obtain direct evidence of conjugate formation between anergic-stimulator or responder-stimulator cells, anergic or potentially responsive cells were labeled with anti-CD3 FITC mAb or its isotypic control, while stimulator cells were incubated with anti-CD14 PE mAb. Conjugate formation at different times after co-incubation of anergic or responder cells with stimulators (at the same ratios employed for in vitro assays) was quantitated by flow cytometry.

Induction of anergy

To obtain anergic cells, 1×10^6 fresh, unstimulated responder PBL or effector cells induced in a primary MLR were incubated with 1×10^6 irradiated (5000 rad) stimulator cells in the presence of anti-B7 mAb, used at a concentration of 10 μg/ml, and CsA (purchased from Sandoz Pharmaceuticals, Basel, Switzerland), employed at a concentration of 400 ng/ml in 24-well flat-bottom plates in 2 ml complete medium. After a 7-day culture, the cells were harvested, tested in a proliferation assay to assess anergy induction, and then employed as suppressor cells in a primary or secondary MLR at a ratio of anergic to responder cells of 3:1, if not otherwise specified.

Mixed lymphocyte reaction

Proliferation of T cells was measured in a one-way MLR using 1×10^5 peripheral blood mononuclear responder cells and 1×10^5 irradiated stimulator cells/well in 96-well round-bottom tissue culture plates in a final volume of 0.2 ml of complete medium. After a 7-day incubation at 37°C in a humidified 5% CO_2 atmosphere, cells were pulsed with 1 μCi/well [^{3}H]TdR (Amersham International, Amersham, UK) and incorporation was measured during the last 21 h by standard procedure (15). T cell proliferation was expressed as the mean cpm of triplicate wells. For the generation of cytotoxic T cells, PBL were cultured in 24-well plates at a concentration of 1×10^6/ml in the presence of 1×10^6 irradiated stimulator cells in 2 ml of complete medium and incubated at 37°C in a humidified 5% CO_2 atmosphere for 7 days.

Proliferation assays

PBL were added in triplicate to flat-bottom microwells at a concentration of 0.5×10^6 cells/ml. PPD (purified protein derivative of tuberculin) (Serum Institute Copenhagen, Denmark) was added at a concentration of 10 μg/ml while tetanus toxoid (TT) (Inalco, Milan, Italy) was employed at a concentration of 5 μg/ml in a final volume of 0.2 ml of RPMI 1640 medium supplemented with 2 mM L-glutamine, 50 μg/ml gentamicin, and 10% human serum. After a 7-day incubation at 37°C in a humidified 5% CO_2 atmosphere, [^{3}H]TdR incorporation was measured by standard procedure. PHA (M-Form, Life Technologies) and OKT3 (Ortho, Raritan, NJ; anti-CD3) activation was obtained by incubating PBL in flat-bottom microwells with PHA (1/20 v/v) and OKT3 mAb (5 ng/ml), respectively.

Cytotoxicity assay

Cytotoxic activity was measured using allogeneic, PHA-activated PBL as target cells as previously described (15). Spontaneous release from the target cells was consistently less than 25%. Results were expressed as percentage of specific lysis.

Data analysis and presentation

Data were stored, analyzed, and reported with the packages STATISTICA/w (StatSoft, Tulsa, OK), and Fig.P (Biosoft, Cambridge, UK), both run on a PowerExec EL (AST, Irvine, CA) personal computer. Results were expressed as mean ± SEM. Normal distribution of data was tested with the Shapiro-Wilk's W test. Differences in means were compared using Student's *t*-test of paired samples. Probability values less than 0.05 were considered to be statistically significant.

Results

In agreement with previous reports (11, 12), the addition of either anti-B7–1 mAb or Cs-A at nontoxic concentrations only partially inhibited proliferation and CTL activity in primary MLR. However, the combination of Cs-A and anti-B7–1 mAb led to nonresponsiveness of alloantigen-induced PBL response (Fig. 1, *a* and *b*). The treatment with Cs-A and anti-B7–1 mAb induced a complete blockage of T cell proliferation, the mean inhibition in 10 experiments being 90% ± 1 (SEM) (*t*-test of paired samples: $p < 0.0005$). PBL activation in a primary MLR was confirmed by the increased expression of surface p55 IL-2R, p75 IL-2R, and class II MHC, and by secretion of IL-2 in the supernatant of the cultures. After addition of anti-B7–1 and Cs-A to the primary MLR a marked decrease in the expression of these molecules and a complete inhibition of IL-2 production was observed, as a result of their inhibitory effect on the activation of PBL (Table I).

We subsequently tested whether cells treated according to this procedure had immunoregulatory effects on alloantigen-specific T cell function. Human lymphocytes primed in a MLR using irradiated human PBL as stimulators were rendered nonresponsive by the addition of anti-B7–1 and Cs-A, and then added to a primary MLR of autologous responder lymphocytes against the same stimulator cells. Figure 1*c* shows that under the experimental condition of a 7-day primary MLR anergic cells were able to induce a complete inhibition of lymphocyte proliferation. The mean inhibitory capacity in eight experiments performed was 74% ± 6 (SEM) (*t*-test of paired samples: $p < 0.001$). Likewise, alloantigen-induced CTL generation could be suppressed by the addition of anergic cells (Fig. 1*d*). These results were not influenced by the use of irradiated (5000 rad) or nonirradiated anergic cells (Fig. 1, *c* and *d*); therefore, in all further experiments anergic cells were used nonirradiated, if not otherwise specified. Addition of the same irradiated stimulator cells to anergic cell culture led to scarcely detectable proliferation (Fig. 1*c*), thus confirming previously reported data on nonresponsiveness of anergic T cells in a secondary MLR (11, 12).

The suppressive effect was further defined in a titration assay in which to a fixed number of responder cells (R) decreasing numbers of stimulators (S) and nonirradiated anergic cells (A) were added (Table II). Results show that the strong suppressive effect elicited was not influenced by the number of anergic cells added to the MLR. In fact, a small number of anergic cells such as that added in the R:A 1:0.01 condition (1000 cells) is able to cause a complete inhibition of MLR, and anergic cells fail to suppress the response only when added at a R:A ratio of 1:0.003.

Interestingly, addition of anergic cells not only prevented alloantigen-induced response during a primary MLR but also inhibited further T cell proliferation in a secondary MLR performed with the same stimulators, in the absence of anergic cells (Table III), indicating the persistence of nonresponsiveness. Moreover, anergic cells could also be generated in a secondary MLR. The cells thus obtained were capable of suppressing proliferation (Table III) and CTL generation (data not shown) of a primary MLR of autologous responder lymphocytes.

The suppressive effect elicited by anergic cells was not restricted to a MLR between autologous responder lymphocytes and the same stimulators used to induce them, because addition of anergic cells to a third party MLR caused the same degree of inhibition observed in the autologous MLR (Fig. 2*a*). Therefore, it was of interest to verify whether anergic cells could exert a suppression on the proliferative response to T lymphocyte polyclonal activators or on specific T cell response to recall Ags. For this purpose, PBL were assayed in the presence of PHA, anti-CD3 mAb, TT and PPD and cocultured with irradiated or nonirradiated autologous anergic cells. Addition of irradiated anergic cells caused a 20% inhibition of both PHA and anti-CD3 activation. Nevertheless, due to the fact that nonirradiated anergic cells retained the ability to proliferate, even though to a lesser extent, to the two mitogens, their addition to the cultures produced an augmented proliferative response. On the other hand, T cell proliferation in response to Ags was not

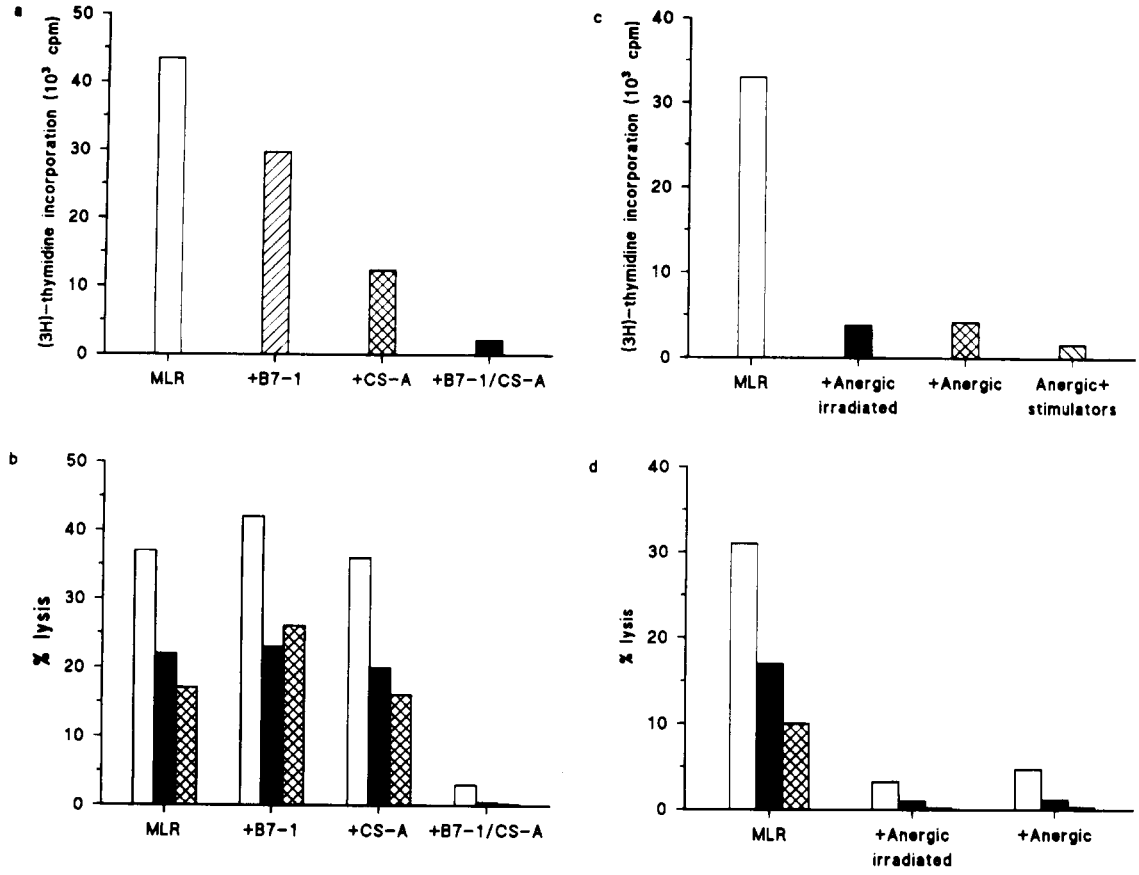

FIGURE 1. Inhibition of alloantigen-induced proliferation (*a*) and cytolytic activity (*b*) of PBL by anti-B7 mAb and/or Cs-A and suppressive effect of alloantigen-induced anergic cells thus obtained on proliferation (*c*) and cytotoxic activity (*d*) of autologous PBL activated in MLR. In *c* and *d*, anergic cells were added either irradiated (■) or not irradiated (▩). Proliferative activity of anergic cells in the presence of stimulator cells was added as control. In *a* and *c*, results are expressed as the mean cpm/minute obtained from triplicate cultures. SEMs were <10%. In *b* and *d*, results are expressed as percentage of specific lysis at an E:T ratio of 100:1 (□), 30:1 (■), and 10:1(▩). Data reported in *a* and *b* are results of one representative experiment out of 10 and 5 MLR-induced proliferation and cytotoxic activity assays, respectively while data reported in *c* and *d* are results of one representative experiment out of 8 and 3 MLR-induced proliferation and cytotoxic activity assays.

Table I. *Surface phenotype and IL-2 production of PBL in a primary MLR and after the addition of Cs-A and anti-B7 MAb BB1*

	MLR	MLR + Cs-A/Anti-B7
Surface Ags[a]		
CD25	67	28
Anti-p75	58	8
Anti-MHC class II	63	18
IL-2 production (U/ml)[b]	12	0

[a] Results are expressed as percentage of positive cells.
[b] Supernatants for the detection of the amount of IL-2 produced were collected after 72 h of culture. IL-2 levels were measured in a CTLL bioassay.

Table II. *Effect of different responder-stimulator (R:S) and responder-anergic (R:A) ratios on T cell proliferation of autologous PBL in a primary MLR*

	R:A Ratio							
R:S Ratio	1:0	1:0.003	1:0.01	1:0.03	1:0.1	1:0.3	1:1	1:3
1:1	30,649[a]	26,820	2,257	2,970	2,077	1,938	1,103	1,044
1:0.5	20,619	20,417	2,017	1,820	1,454	1,819	908	664
1:0.1	9,151	8,889	1,249	2,385	1,302	1,388	906	702

[a] Results are expressed as the mean of triplicate cultures. SEMs were <10%.

affected by coculture with irradiated or nonirradiated anergic cells (Fig. 3).

To determine the mechanism involved in this suppressive phenomenon, we tested whether consumption of IL-2 could account for the inhibitory effect here observed. In opposition to this possibility, exogenous IL-2 added at two different concentrations was not able to restore alloantigen-induced lymphocyte proliferation (Fig. 2*b*). Comparable results were obtained in the setting of a HLA-matched unrelated donor-recipient pair found to be identical for HLA A and B Ags by standard serologic typing and for DR,

Table III. *Effect of the addition of anergic cells, generated in a primary or secondary MLR, on T cell proliferation of autologous PBL in a primary and a secondary MLR*[a]

	Primary MLR	Secondary MLR
Control MLR	21,323[b]	17,391
+ primary MLR anergic cells	4,508	748
Control MLR	23,993	ND
+ secondary MLR anergic cells	1,307	ND

[a] Secondary MLR was performed without further addition of anergic cells.
[b] Results are expressed as the mean of triplicate cultures. SEMs were <10%.

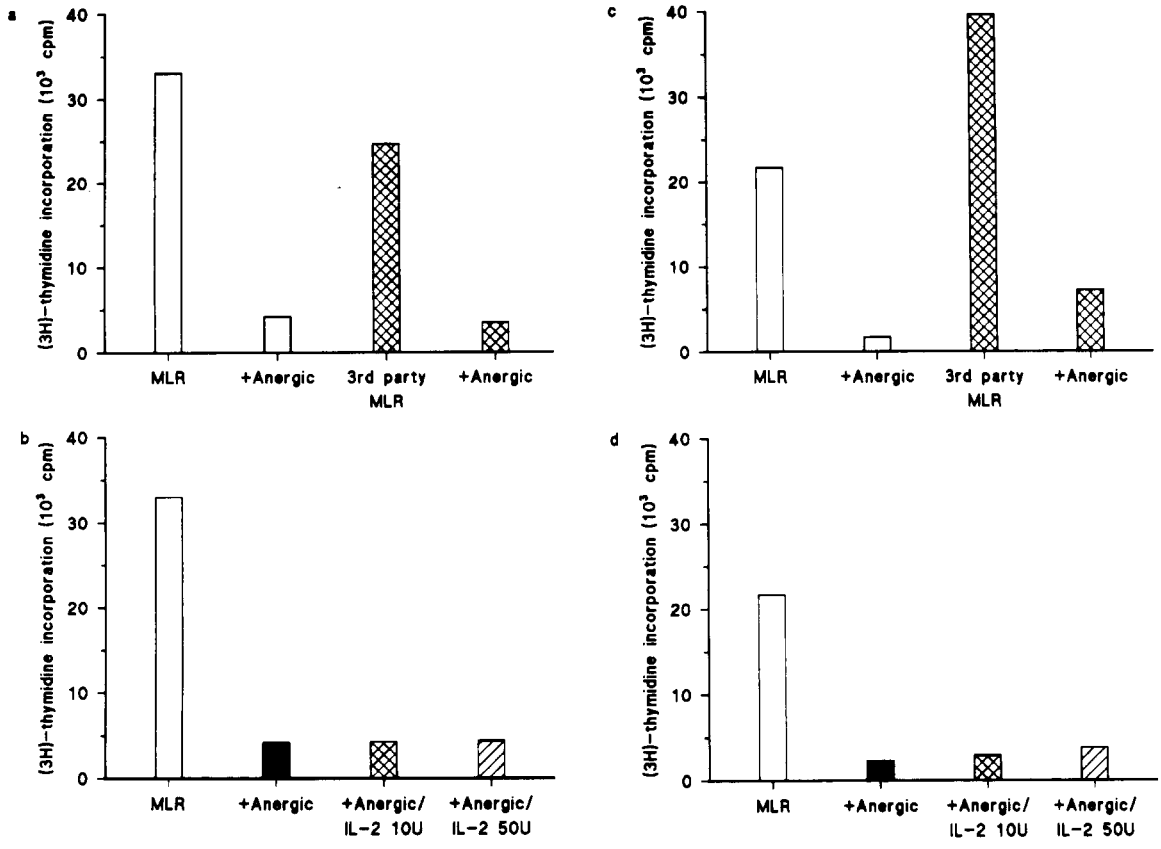

FIGURE 2. Suppressive effect of alloantigen-induced anergic cells on proliferation of autologous PBL activated in MLR. *a* and *b* refer to unrelated, HLA-different donors, while *c* and *d* refer to a HLA-matched unrelated donor/recipient pair. In *a* and *c*, data concerning the suppressive effect of anergic cells on MLR response of autologous PBL against third party stimulators are reported. Donors' HLA is as follows: *a*, responders: A 1, 9(24); C w7, X; B 5(w52), 8; DR 2, 3; DQ w1, w2; stimulators: A 2, 10(26); C X; B 5(51), 16(39); DR 2, 7; DQ w1, w2; third party stimulators: A 3, X; C w4, w7; B 7, 35; DR 1, 2; DQ w1, X. *c*, responders: A 2, 2; B 44, 49; DR 1, 11 (DRβ1 0901/1301; DRβ3 0101; DRβ4 0101); DQ (DQα 0301/0103; DQβ 0303/0603); stimulators: A 2, 2; B 44, 49; DR 1, 11 (DRβ1 0901/1301; DRβ3 0101; DRβ4 0101); DQ (DQα 0301/0103; DQβ 0303/0603); third party stimulators: A 3, 3; C X, w3; B 18, 40(w60); DR 7, 7; DQ w2, w2. In *b* and *d*, the effect of addition of 2 different doses of rIL-2 on suppression elicited by anergic cells is reported. Results are expressed as the mean cpm/min obtained from triplicate cultures. SEMs were <10%.

DQ, and DP Ags by molecular analysis, and thus selected for bone marrow transplantation (Fig. 2, *c* and *d*).

Anergic cells could also exert their suppressive effect by either secreting cytokines inhibitory of reactive T cells or, alternatively, by delivering a direct signal through cell-cell contact. To verify these hypotheses, responders and stimulators of a primary MLR were assayed in 24-well plates and autologous anergic cells were added to the culture separately, by means of transwell permeable supports. In this way, the passage of suppressive factors potentially secreted by anergic cells was allowed; however, the microporous membrane hindered any contact between the anergic cells and responders or APC surface. Under these conditions, anergic cells were not able to inhibit alloantigen-specific lymphocyte proliferation (Fig. 4). Furthermore, anergic cells were assayed in the permeable chamber in the presence of irradiated stimulators, to evaluate the possibility that a contact between anergic cells and APC was responsible for the secretion of soluble suppressive factors. Again, no inhibition was observed, indicating that this suppressive phenomenon could only be accounted for by the delivery of a signal through cell-cell contact. In support to this hypothesis, we verified the presence of conjugate formation between anergic or potential responder cells (fresh PBL) and the same stimulators used to induce anergy or third party stimulators. Conjugate

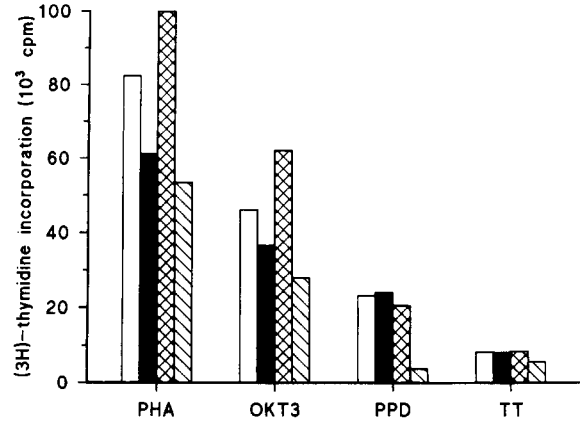

FIGURE 3. Effect of anergic cells on proliferative response of autologous lymphocytes to T cell mitogens or recall Ags. Proliferative response in the absence of anergic cells (□), and in the presence of irradiated (■) or not irradiated (▩) cells is reported. Proliferative response of anergic cells in the presence of the same stimulators is also shown (▧). Proliferation of responders and anergic cells in medium alone was always less than 3×10^2 cpm. Results are expressed as the mean cpm/minute obtained from triplicate cultures. SEMs were <10%.

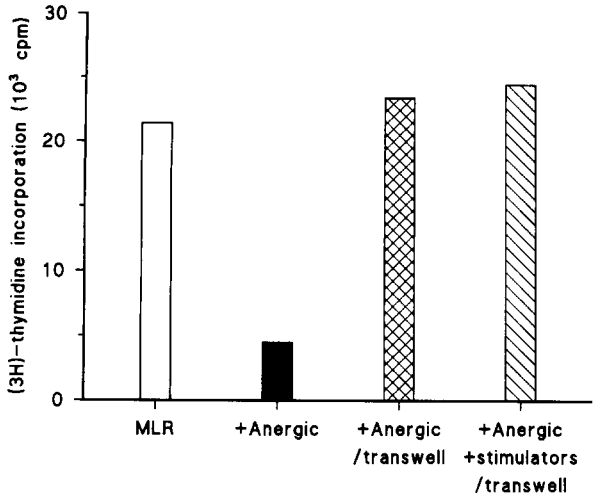

FIGURE 4. Evaluation of suppressive effect of anergic cells on proliferation of autologous PBL activated in MLR when cell to cell contact between anergic cells and responders+stimulators is hindered by means of transwell permeable support assay (▩). PBMC were assayed in 24-well plates (Costar, Cambridge, MA) at a concentration of 5×10^5/ml in the presence of 5×10^5 irradiated stimulators in a final volume of 1 ml (lower chamber). 1.5×10^6 autologous anergic cells were added separately to the culture by means of a porous cell culture insert (pore size 0.4 μm) (Transwell permeable support, Costar). A control in which irradiated stimulators were assayed with anergic cells in the permeable chamber was also performed (▧). Results are expressed as the mean cpm/min obtained from triplicate cultures. SEMs were <10%.

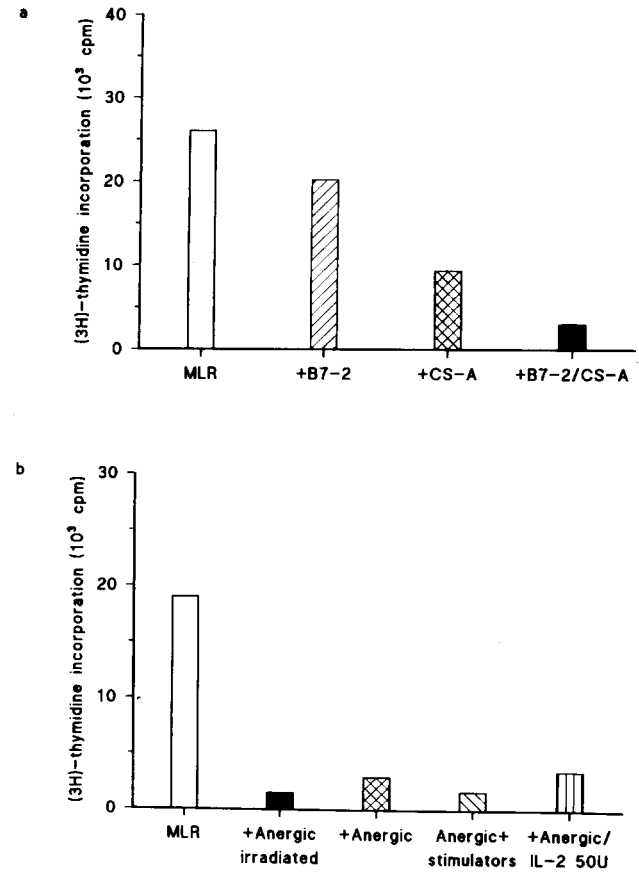

FIGURE 5. Inhibition of alloantigen-induced proliferation (*a*) of PBL by anti-B7–2 mAb and/or Cs-A and suppressive effect of alloantigen-induced anergic cells thus obtained on proliferation (*b*) of autologous PBL activated in MLR. Anergic cells were added either irradiated (■) or not irradiated (▩). Proliferative activity of anergic cells in the presence of stimulator cells was added as control. The effect of addition of rIL-2 (50 U/ml) on suppression elicited by anergic cells is also reported. Results are expressed as the mean cpm/minute obtained from triplicate cultures. SEMs were <10%. Data reported in *a* and *b* are results of one representative experiment out of 2 MLR-induced proliferation.

formation was evaluated by dual parameter FACS analysis measuring CD3 FITC and CD14 PE double-positive populations on gated CD14+ cells. The number of conjugates between anergic cells and stimulators was higher (original stimulators: 97%; 3rd party stimulators: 92%) than that observed between potential responders and stimulators (original stimulators: 58%; 3rd party stimulators: 55%) after a 15-min incubation. Similar results were obtained at 30 and 45 min.

Finally, we preliminary investigated the possible role of another member of B7 molecule family, namely CD86 (B7–2), on the generation of both alloantigen-induced anergy and suppressive effect. Data reported in Figure 5 indicate that the combined use of anti-B7–2 mAb and Cs-A, in the same culture conditions employed for anti-B7–1 assays, gave results comparable with those observed with anti-CD80 mAb. In fact, a state of nonresponsiveness was also induced by addition of anti-CD86 mAb and Cs-A, and the anergic cells thus obtained were able to suppress a primary MLR of autologous responder cells. The addition of IL-2 to the cultures did not restore alloantigen-induced proliferation. Moreover, similar results were also attained by employing both anti-B7–1 and B7–2 mAb in the assay (data not shown).

Discussion

Our results demonstrate that alloantigen-induced PBL rendered nonresponsive by addition of a combination of anti-B7–1 mAb and CsA to a primary or secondary MLR can exert an inhibitory effect on autologous nonanergic PBL by blocking their proliferative and cytolytic activity in a MLR. Moreover, they also suppress further PBL proliferation in a secondary MLR performed with the same stimulators, in the absence of anergic cells. These data confirm and extend those reported previously by Van Gool et al., who provided compelling evidence on the synergistic blocking effect of CsA and anti-B7 mAb on T cell activation (11, 12, 16). Furthermore, they suggest that these anergic cells are not neutral, but may propagate anergy to potentially responsive T lymphocytes. The idea of "spreading or creeping anergy" was initially proposed by Waldmann et al. (17) and recently confirmed by the experiments reported by Lombardi et al. (13) on suppressor capacity of human Ag-specific anergic T cell clones. In the latter study, T cell anergy was induced by incubating T cell clones with specific peptide or immobilized mAb to CD3 in the absence of any added APC, according to previously described protocols (18–20). Our experiments show that anergic alloantigen-induced PBL with suppressive activity can also be obtained by blocking in vitro the CD28/B7 costimulation pathway by means of anti-CD80 mAb or, as inferred from preliminary experiments, anti-CD86 mAb, in the presence of low Cs-A concentrations (11, 12).

At difference with the data reported by Lombardi et al. (13), we found that the suppressive effect mediated by the anergic alloantigen-induced PBL here described could not be reversed by addition of exogenous IL-2, and a low percentage of anergic cells expressed IL-2R. These observations suggest that consumption of IL-2 is unlikely to be the cause of inhibition.

The experiment performed by means of transwell permeable supports, which allowed the physical separation of responder and stimulator cells of the MLR from anergic cells, demonstrated that the suppressive effect can be elicited only when there is a cell to cell contact between the three populations. These results suggested either a competition between anergic and nonanergic lymphocytes for stimulator cell surface or the delivery of a signal mediated by anergic cells through a cell-cell contact as possible mechanisms responsible for the inhibitory effect. The data obtained from the titration assay of anergic and stimulator cells strongly support the second hypothesis.

The suppressive activity mediated by anergic cells on alloantigen-induced proliferation of autologous PBL was not restricted to their response against those alloantigens employed to induce anergy, since reactivity to a third party stimulator was also inhibited. Nevertheless, alloantigen-induced anergic PBL maintain the capacity to proliferate in response to both T lymphocyte polyclonal activators and recall Ags, and cause only a slight inhibition of the proliferation of autologous nonanergic PBL to the same activators, thus showing that the suppressive effect is only limited to alloreactivity.

Most of the reported experiments have been performed with responder and stimulator cells obtained from unrelated subjects with profound HLA disparity. However, similar results were observed using a HLA-matched unrelated donor-recipient pair identical for HLA-A, -B, -DR, -DQ, and -DP Ags who was selected for bone marrow transplantation. Data concerning this donor/recipient pair demonstrate that alloantigen-induced anergy and suppressor activity obtained in vitro by combined treatment with anti-B7–1 mAb and low doses of Cs-A can also be achieved when responder and stimulator cells share the majority of HLA Ags, and low levels of lymphocyte proliferation in MLR is observed.

Taken together, the results of our study provide in vitro evidence that alloantigen-induced PBL rendered anergic by treatment with Cs-A and anti-CD80 mAb can induce nonresponsiveness of autologous PBL activated in MLR, thus propagating the state of tolerance toward alloantigens, but not recall Ags. The description of this phenomenon, together with previously reported data (11–13) suggests that this procedure might contribute to the planning of new strategies aimed at promoting and mantaining prolonged alloantigen-specific tolerance in vivo. The combined use of anti-B7 mAb and low doses of Cs-A could prove to be of relevant interest in the setting of bone marrow transplantation, especially for transplants performed using HLA-partially matched family donors or HLA-matched unrelated donors, conditions in which, due to the antigenic disparity between donor and recipient, the induction of a tolerance state is particularly difficult to obtain.

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