Costimulatory Molecule Requirement for Bovine WC1+ yo T Cells' Proliferative Response to Bacterial Superantigens

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ABSTRACT

We have previously shown that the proliferation of freshly isolated bovine WC1+ yo T cells to superantigens (SAgs) including staphylococcal enterotoxin A (SEA), and staphylococcal enterotoxin B (SEB) or toxic shock syndrome type-1 (TSST-1) required the presence of antigen-presenting cells (APC) and the addition of exogenous interleukin (IL)-2. The costimulatory activity provided by molecules expressed on professional APC for the proliferation of yo T cells has not been addressed hitherto. In the present study, we investigated the ability of two selected APC populations, the dendritic cells (DCs) highly expressing CD80 and CD86 molecules (CD80highCD86high) and the monocytes expressing the same molecules at a rather low level (CD80lowCD86low), to stimulate the proliferation of purified bovine WC1+ yo T cells to SAgs. DCs were more efficient than monocytes in inducing yo T-cell proliferation, and this response was dependent on exogenous IL-2 in both presentation modes. Stimulating yo T cells with gradual doses of SAgs or concanavalin A (ConA) resulted in similar dose-dependent reaction profiles suggesting a minimal role of the major histo-compatibility complex (MHC). However, significant proliferation was already obtained with the starting doses in the presence of DC compared with monocytes, and higher proliferation was reached with DC at optimal doses. Finally, the addition of monoclonal antibody (MoAb) anti-CD86 markedly inhibited SAgs- and ConA-mediated proliferation, whereas MoAb anti-CD80 had no effect. The combination of both anti-CD80 and anti-CD86, however, suppressed this response. These results suggest that bovine yo T-cell proliferation response requires indubitably CD86 costimulation. The role of CD80 molecule seems less clear.

INTRODUCTION

T-cell activation and proliferation require two signals. One signal delivered via the T-cell receptor (TCR) is major histocompatibility complex (MHC)-restricted and antigen-specific. The other signal is delivered by costimulatory molecules of professional antigen-presenting cells (APC) and is nonspecific. Two structurally related molecules expressed on the surface of T cells, CD28 and cytotoxic T-lymphocyte-associated molecule (CTLA-4), and their coreceptors CD80 and CD86 expressed on a variety of professional APC.
including dendritic cells (DCs), activated macrophages and activated B cells, are the best-characterized costimulatory molecules identified to date. CD28 and CTLA-4 costimulatory molecules have also been detected on bovine and ovine yo T cells. However, few studies have provided information concerning the costimulatory activity required for the activation of ruminant yo T cells.

Most of the circulating bovine and ovine yo T cells express a unique family of high molecular weight surface mol-ecules, termed workshop cluster 1 (WC1). These cells constitute up to 30-50% in the peripheral blood in young ruminants, and are further characterized by a CD2-, CD4-, CD8-, CD3+ and TCR yo+ phenotype.

The absolute requirement of APC and exogenous inter-leukin (IL)-2 for the proliferation of purified yo T cells in vitro upon a stimulation with mitogens, specific antigen and recently with superantigens (SAgs) has been repeatedly reported.

In order to assess the role of the costimulation provided by molecules expressed on APC, we analysed the proliferation of purified yo T cells to SAgs in the presence of two professional APC populations expressing different levels of CD80 and CD86 molecules: the DC and the monocytes. The respective role of either ligand was studied using blocking antibodies to CD80 and CD86.

MATERIALS AND METHODS

Reagents. The culture medium contained RPMI 1640, 2 mM L-glutamine, 25 mM HEPES buffer, 100 IU/ml penicillin, 100 µg/ml streptomycin, 0.5 µg/ml fungizone and 10% foetal calf serum (FCS) (Gibco BRL, Grand Island, NY, USA). The murine monoclonal antibodies (MoAbs) CC8 (immunoglobulin, IgG2a), CC63 (IgG2a), CC15 (IgG2a), CC-G33 (IgG1), ILA21 (IgG2a) and ILA30 (IgG1)
specific for CD4, CD8, WC1, CD14, MHC-II and IgM, respectively, were purchased from SeroTec Immunological (Oxford, UK). The murine MoAbs BB1 (IgM) and IT2.2 (IgG2b) specific for human CD80 and CD86, respectively, were purchased from Pharmingen (San Diego, CA, USA). Isotype-matched mouse IgM (M 5909), mouse IgG2b (M 5534) and mouse IgG2a (M 5409) were from Sigma Immunochemicals (St. Louis, MO, USA). Staphylococcal enterotoxin A (SEA), staphylococcal enterotoxin B (SEB), toxic shock syndrome type-1 (TSST-1), concanavalin A (ConA), calcium ionophore A and phorbol myristate acetate (PMA) were from Sigma Immunochemicals. Recombinant human IL-2 was from Boehringer (Engelheim, Germany). Lymphoprep and Nycodenz gradients (d = 1.068) were from Nycomed Pharma (Oslo, Norway). Calcium ionophore was dissolved in dimethyl sulphoxide (DMSO) and PMA in anhydrous ethanol at a concentration of 1 mg/ml and 10 µg/ml, respectively. Purification of bovine WC1+ yo T cells, WC1+ yo T cells were negatively purified from peripheral blood as previously described [8]. Using Lymphoprep, peripheral blood mononuclear cells (PBMCs) were isolated from heparinized peripheral venous blood from 6- to 12-month-old cattle. PBMCs at 5 x 106 cells/ml were resuspended in culture medium and allowed to adhere for 60 min to plasma-coated gelatin. Nonadherent cells were enriched in T cells by passing the cells through a nylon wool column to remove B cells and remaining monocytes. CD2+ T cells were
depleted by rosetting T cells with 2-aminoethylisothiouronium bromide (AET)-treated sheep red blood cells (SRBC). WC1+ yo T cells were further separated from CD4+ and CD8+ T cells using the magnetic cell sorting (MACS) technique. The resultant population contained up to 97% of WC1+ yo T cells as stated by the fluorescence-activated cell sorter (FACS) phenotypic characterization of cells. Isolation of bovine DC and monocytes. DC (CD14-MHC-IIhigh) and monocytes (CD14+) were purified as described by Renjifo et al. PBMCs at 5 x 10^5 cells/ml were allowed to adhere for 2 h to plasma-coated gelatin. Nonadherent cells were removed by washing three times with Hank's balanced salt solution (HBSS). Cells adhered to the gelatin were detached by incubating for 5 min with prewarmed HBSS containing 0.2% ethylenediaminetetraacetic acid (EDTA), washed three times, and cultured overnight. Plastic adherent cells were monocytes, while the supernatant contained nonadherent cells rich in DCs. DCs were further enriched by centrifugation in Nycodenz gradients (d = 1.066), washed three times, and separated from CD14+ and IgM+ cells using the MACS technique. Flow cytometry analysis. Cells, 10^6 per test, were washed twice with ice-cold phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA), incubated on ice for 30 min with an appropriate dilution of MoAb, washed in PBS, and further incubated for 30 min on ice with fluorescein isothiocyanate (FITC)-labelled MoAb specific for kappa chain as the second reagent. After washing, phenotypic analysis was performed on FACS flow cytometer (FACScalibur; Becton-Dickinson, Mountain View, CA, USA). Proliferation assays. PBMCs (2 x 10^5 cells in 0.2 ml) were stimulated in triplicate with ConA (1 µg/ml), SEA (103 ng/ml), and PMA and/or ionomycin (40 ng/ml) for 4 days. Purified WC1+ yo T cells (2 x 10^5 cells) and autologous y-irradiated APC (5000 Rad of gamma-irradiation from a 137Cs source) were cultured in 0.2 ml in triplicate with SEA, SEB, TSST-1 or ConA for 4 days. When indicated, the recombinant human IL-2 was added at a final concentration of 50 units/ml. The cultures were pulsed for the final 18 h with 1 µCi of [3H]-thymidine, harvested and the incorporated radioactivity determined by liquid scintillation counting. To assess the effect of anti-CD80 and anti-CD86 on yo T-cell proliferation, MoAbs were added to DC for 30 min before the beginning of the culture period at a predetermined dose (1 µg/ml). Statistical analysis. The results were expressed as the mean ± standard deviation (SD) and compared using a Student's t-test. The significance level was set at P < 0.05.

RESULTS

Flow cytometry analysis of enriched DC and monocytes Monocytes and DCs are characterized by a high CD14+ expression and a lack of CD4+ expression, respectively, and by MHC-II levels, high in DC and low in monocytes (Fig. 1). Flow cytometry analysis after purification shows 89% of the cells expressing CD14+/MHC-IIlow, representing monocytes (Fig. 1A,C), and 85% of the cells, CD14 MHC-II, being DC (Fig. 1B,C). DCs also express CD80 and CD86 molecules at a higher level than the monocytes (Fig. 1D,E). Enriched DC contained <1% monocytes, <1% B cells, and T cells including " 4% WC1+, " 5% CD4+ and " 3% CD8+ (FACS not shown). DC and monocyte antigen presentation needs the addition of exogenous IL-2 for the proliferation of WC1+ yo T cells to SAgS and ConA Isolated WC1+ yo T cells cultured with or without auto- logous y-irradiated DC or monocytes were pulsed in various combinations with SAgS (SEA, SEB or TSST-1), ConA and IL-2 (Table 1). The yo T cells responded to all SAgS and ConA in the
presence of DC or monocytes and IL-2, but failed to respond in the presence of either stimulus and/or IL-2 in the absence of APC. The amplitude of the responses to similar stimuli was significantly higher with DC than monocytes ($P < 0.01$).

![Flow cytometry analysis of bovine dendritic cells (DCs) and monocytes. Monocytes (A) and DCs (B) were stained with CC-G33 monoclonal antibody (MoAb) specific for CD14 and fluorescein isothiocyanate (FITC)-labelled MoAb specific for kappa chain as secondary reagent. The indicated antigen-presenting cell (APC) populations were stained with IIA21 (C), BB1 (D) and IT2.2 (E) MoAb specific for major histocompatibility complex (MHC)-II, CD80 and CD86, respectively, and FITC-labelled MoAb specific for kappa chain. Negative controls consisting of APC populations were stained with FITC-labelled MoAb specific for kappa chain alone. (C-E) Controls staining for both APC (DC and monocytes) did not vary significantly. All stainings were performed in the presence of 10% foetal bovine serum (FBS) in order to inhibit Fc receptor (FcR) binding of staining reagents.]

*Fig. 1. Flow cytometry analysis of bovine dendritic cells (DCs) and monocytes. Monocytes (A) and DCs (B) were stained with CC-G33 monoclonal antibody (MoAb) specific for CD14 and fluorescein isothiocyanate (FITC)-labelled MoAb specific for kappa chain as secondary reagent. The indicated antigen-presenting cell (APC) populations were stained with IIA21 (C), BB1 (D) and IT2.2 (E) MoAb specific for major histocompatibility complex (MHC)-II, CD80 and CD86, respectively, and FITC-labelled MoAb specific for kappa chain. Negative controls consisting of APC populations were stained with FITC-labelled MoAb specific for kappa chain alone. (C-E) Controls staining for both APC (DC and monocytes) did not vary significantly. All stainings were performed in the presence of 10% foetal bovine serum (FBS) in order to inhibit Fc receptor (FcR) binding of staining reagents.*

DC induced high proliferative response of WC1+ yo T cells to SAgS and ConA We next compared the ability of DC and monocytes to induce yo T-cell response in vitro. The proliferation of IL-2-dependent WC1+ yo T cells to SAgS or ConA in the presence of DCs or monocytes is depicted in Fig. 2. The response of purified yo T cells to SAgS and ConA was observed to decrease with the decreasing APC populations. In the presence of DC, wells containing 2 x 10^4 DCs, at a ratio of 1:10 yo T cells, induced "43 x 103,"30 x 103,"31 x 103 and "27 x 103 counts per minute (cpm) for ConA, SEA, SEB and TSST-1, respectively. Monocytes at the same ratio induced "22 x 103,"11 x 103,"12 x 103 and "9 x 103 cpm for ConA, SEA, SEB and TSST-1, respectively. At the ratio of 1 DC (2 x 10^3): 100 yo T cells, the magnitude of the proliferation was "21 x 103,"15 x 103,"16 x 103 and "13 x 103 cpm for ConA, SEA, SEB and TSST-1, respectively, in the presence of
DC, whereas proliferation responses equivalent to controls were obtained using monocytes. Dose-dependent proliferative response of purified WC1+ yot cells to SAGs and T-cell mitogens in the presence of DC or monocytes The proliferation of purified WC1+ yot T cells stimulated with increasing doses of SAGs and ConA in the presence of constant number of DC or monocytes and IL-2 is shown in Fig. 3. The yot T cells responded to minute amounts of SAGs and ConA in the presence of DC, while monocytes required high doses of stimulus to induce detectable levels of yot T-cell response. At the same antigen or mitogen doses, yot T-cell proliferation was consistently of higher magnitude with DC in comparison with monocytes. Control yot T-cell cultures with increasing doses of stimulus and IL-2 without APC did not proliferate.

Table 1. The proliferative response of WC1+ yot T cells to supernantens (SAGs) and concanavalin A (ConA) in the presence of professional antigen-presenting cell (APC) populations

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Without APC (cpm)</th>
<th>γ-Irradiated APC (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Monocytes</td>
</tr>
<tr>
<td>Median</td>
<td>1025 ± 280</td>
<td>991 ± 179</td>
</tr>
<tr>
<td>IL-2</td>
<td>1235 ± 365</td>
<td>974 ± 247</td>
</tr>
<tr>
<td>SEA</td>
<td>989 ± 236</td>
<td>1034 ± 239</td>
</tr>
<tr>
<td>SEB</td>
<td>1135 ± 185</td>
<td>1163 ± 228</td>
</tr>
<tr>
<td>TSST-1</td>
<td>908 ± 276</td>
<td>978 ± 241</td>
</tr>
<tr>
<td>ConA</td>
<td>805 ± 254</td>
<td>1106 ± 189</td>
</tr>
<tr>
<td>SEA + IL-2</td>
<td>925 ± 330</td>
<td>1209 ± 1932†</td>
</tr>
<tr>
<td>SEB + IL-2</td>
<td>1005 ± 210</td>
<td>14123 ± 1729†</td>
</tr>
<tr>
<td>TSST-1 + IL-2</td>
<td>825 ± 154</td>
<td>12979 ± 1937†</td>
</tr>
<tr>
<td>ConA + IL-2</td>
<td>1204 ± 199</td>
<td>15821 ± 2937†</td>
</tr>
</tbody>
</table>

*Purified WC1+ yot cells (2 x 105/well) were cultured in triplicate in the presence or absence of γ-irradiated autologous APC (2 x 104 cells/well) and stimulus, and the proliferation was assessed by [3H]-thymidine incorporation during the last 18 h of 4 days of culture. The results represent the mean counts per minute (cpm) ± standard deviation (SD) of three independent experiments from three animals. Interleukin (IL)-2 was added at a final concentration of 50 units/ml, SAG at 103 ng/ml and ConA at 10 μg/ml.

†P < 0.01 (significant difference between DC and monocytes).

Fig. 2. Proliferative response of WC1+ yot T cells to supernantens (SAGs) and concanavalin A (ConA) in the presence of decreasing number of professional antigen-presenting cells (APC). WC1+ yot T cells (2 x 105/well) were cultured in triplicate with decreasing number of autologous γ-irradiated dendritic cells (DCs) or
monocytes in the presence of streptococcal enterotoxin A (SEA), streptococcal enterotoxin B (SEB) or toxic shock syndrome type-1 (TSST-1) (103 ng/ml) or ConA (10 μg/ml), and interleukin (IL)-2 (50 U/ml). Proliferation was assessed by thymidine incorporation during the last 18 h of 4 days of culture. The results are expressed as counts per minute (cpm), and each point represents the mean ± standard deviation (SD) from three independent experiments.

Blocking of bovine DC costimulatory molecules with human MoAbs anti-CD80 and anti-CD86

Preliminary experiments were devised in order to establish the activity of MoAbs anti-CD80, anti-CD86 and anti-MHC-II in the inhibition of bovine T-cell proliferation in vitro. PBMCs were stimulated with SAgS or T-cell mitogens (ConA, PMA and/or mitomycin) in the presence of anti-odies to CD80, CD86, MHC-II, combination of CD80 and CD86, or combination of CD86 and MHC-II. Isotype- matched mouse IgM, mouse IgG2a and mouse IgG2b were used as controls. Table 2 shows that the anti-CD86 but not anti-CD80 markedly inhibited SEA-mediated T-cell prolif-eration (P < 0.05), the combination of both anti-CD80 and anti-CD86 resulting in more significant inhibition (P < 0.01).

Anti-MHC-II alone or in combination with anti-CD86 sup-presses completely the proliferation (P < 0.01). The addition

![Diagram](image)

Fig. 3. Proliferative response of WC1+ yo T cells to gradual doses of superantigens (SAgs) and concanavalin A (ConA) in the presence of dendritic cells (DCs) or monocytes. WC1 + yo T cells (2 x 10⁵/well) were cultured in triplicate with or without autologous γ-irradiated DC or monocytes (2 x 10⁴ cells/well), in the presence of gradual doses of streptococcal enterotoxin A (SEA) (A), streptococcal enterotoxin B (SEB) (B), toxic shock syndrome type-1 (TSST-1) (C) or ConA (D) with or without interleukin (IL)-2 addition (50 U/ml). Proliferation was assessed by thymidine incorporation during the last 18 h of 4 days of culture. The results are expressed as counts per minute (cpm), and each point represents the mean ± standard deviation (SD) from three independent experiments.
of anti-CD86 alone significantly inhibits ConA-mediated T-cell proliferation (P < 0.01), while anti-CD80 alone had no effect. A combination of both anti-CD80 and anti-CD86 strongly inhibited the proliferation induced by ConA (P < 0.01). In contrast to SAg, ConA-mediated T-cell response was not inhibited by the addition of anti-MHC-II. All combinations of isotype-matched controls remained ineffective. The specificity of the anti-CD80, anti-CD86 and anti-MHC-II-mediated inhibition was further verified by analysis of the blocking properties of the MoAbs in the costimulatory-independent response. As shown in Table 2, the proliferation response induced by PMA + ionomycin was not inhibited by MoAbs to CD80, CD86, MHC-II, the combination of CD80 and CD86 or the combination of CD86 and MHC-II. PMA or ionomycin alone did not induce the proliferation of PBMC (not shown). To study the role of the DC costimulatory molecules on IL-2-dependent yo T-cell response, purified cells were cultured with SAgS SEA (Fig. 4A), SEB (Fig. 4B) or TSST-1 (Fig. 4C), or with ConA (Fig. 4D), in the presence of anti-CD80 and/or anti-CD86 antibodies. Anti-CD86, but not anti-CD80, significantly inhibited the proliferation of WC1+ yo T cells in response to all SAgS tested and to ConA. The combination of both MoAbs almost completely suppresses the proliferative response. All isotype-matched MoAb controls remained ineffective.

**DISCUSSION**

The costimulation requirement for bovine yo T-cell proliferative response constitutes the aim of this study. We previously reported that the proliferation of purified bovine WC1+ yo T cells, in response to bacterial SAgS (SEA, SEB and TSST-1) and T-cell mitogen (ConA), required the presence of APC and the addition of exogenous IL-2 [18]. The same observations have also been recorded with specific antigen [17]. To investigate how accessory cells mediate the proliferation of yo cells, we compared the efficiency of DC and monocytes to induce this response. DCs are APC with a strong capacity to stimulate T lymphocytes, owing to their high density of MHC-II and costimulatory molecules [23]. Monocytes are also APC-identifed by the low density of both MHC-II and costimulatory molecules. Although DC and monocytes were able to present SAgS and induce yo T-cell proliferative response in the presence of exogenous IL-2, they showed a marked difference in their efficiency. The DC peripheral blood mononuclear cells (PBMCs) (2 x 105 cells/well) were incubated with monoclonal antibodies (MoAbs) anti-CD80, anti-CD86 and/or anti-MHC-II at a final concentration of 1 μg/ml and stimulated in vitro by concanavalin A (ConA) (10 μg/ml), staphylococcal enterotoxin A (SEA) (103 ng/ml) or combination of phorbol myristate acetate (PMA) (40 ng/ml) and ionomycin (40 ng/ml), and the proliferation was evaluated by [3H]-thyidime incorporation during the last 18 h of 4 days of culture. Isotype-matched mouse immunoglobulin (IgM) mouse IgG2b and mouse IgG2a were used as controls.
Table 2. Specificity of anti-CD80, anti-CD86 and anti-major histocompatibility complex (MHC)-II-mediated inhibition of bovine T-cell proliferation in vitro.

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>PBMC (cpm)</th>
<th>SEA</th>
<th>ConA</th>
<th>PMA + ionomycine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium</td>
<td>23780 (909)</td>
<td>63328 (1330)</td>
<td>72269 (2172)</td>
<td></td>
</tr>
<tr>
<td>Anti-CD80</td>
<td>29098 (1391)</td>
<td>63000 (818)</td>
<td>76094 (455)</td>
<td></td>
</tr>
<tr>
<td>Anti-CD86</td>
<td>11080 (1375)*</td>
<td>34288 (1699)**</td>
<td>72174 (2019)</td>
<td></td>
</tr>
<tr>
<td>Anti-CD80 + Anti-CD86</td>
<td>4097 (393)**</td>
<td>12603 (559)**</td>
<td>73008 (221)</td>
<td></td>
</tr>
<tr>
<td>Anti-MHC-II</td>
<td>1208 (237)**</td>
<td>61671 (762)</td>
<td>71472 (1725)</td>
<td></td>
</tr>
<tr>
<td>Anti-MHCII + Anti-CD86</td>
<td>1088 (77)**</td>
<td>34726 (464)**</td>
<td>72174 (2019)</td>
<td></td>
</tr>
<tr>
<td>IgM</td>
<td>24287 (398)</td>
<td>61987 (1306)</td>
<td>71679 (1697)</td>
<td></td>
</tr>
<tr>
<td>IgG_2b</td>
<td>24120 (1282)</td>
<td>61933 (1436)</td>
<td>72263 (2473)</td>
<td></td>
</tr>
<tr>
<td>IgG_3b</td>
<td>23612 (519)</td>
<td>62522 (1159)</td>
<td>71529 (1342)</td>
<td></td>
</tr>
<tr>
<td>IgM + IgG_2b</td>
<td>24003 (1846)</td>
<td>62918 (1116)</td>
<td>76285 (1041)</td>
<td></td>
</tr>
<tr>
<td>IgG_3b + IgG_2b</td>
<td>23510 (2085)</td>
<td>63100 (1207)</td>
<td>72637 (2392)</td>
<td></td>
</tr>
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</table>

Data represent mean counts per minute (cpm) and standard deviation (SD) (in parentheses) of triplicate cultures. Proliferation of PBMC in the presence of medium alone was 852 ± 102 cpm. *P < 0.05; **P < 0.01 - significant difference between SEA or ConA-mediated T-cell response in the absence or presence of indicated monoclonal antibodies. Induced a much stronger yo T-cell response compared with monocytes. The usefulness of the addition of IL-2 to the cultures to induce the proliferation of yo T cells has been documented by many authors. WC1+ yo T cells have been found to express the gene for IL-2R [8, 17] but not for IL-2 on SAg-activated cells [18], indicating the need of exogenous IL-2 addition to SAg- and ConA-mediated yo T-cell proliferation. We and others have previously reported that uncultured WC1+ yo T cells negatively purified (97%) expressed CD28 and CTLA-4 mRNA. The interaction of CD80 and CD86 on APC with CD28 and CTLA-4 on T cells has been recognized as being of major importance in the stimulation of T-cell responses. In addition, the level of costimulatory molecules and MHC antigen expression on APC has been suggested to reflect the amplitude of the T-cell response to SAg. Bovine DC and monocytes differ markedly in the expression of CD80, CD86 and MHC-II molecules, as shown in the FACS results. The efficiency of DC (MHC-IIIhigh, CD80high and CD36high), compared with monocytes (MHCIIIlow, CD80low and CD86low), in inducing WC1+ yo T-cell response to SAg could, therefore, be related to the level of MHC-II antigen expression and/or to the expression of the costimulatory molecules. Using increasing doses of ConA and SAg to stimulate purified yo T cells in the presence of either DC or monocytes resulted in different dose-dependent proliferation levels. The monocytes required higher doses of stimulus to induce detectable proliferative response of yo T cells to both SAg and ConA. In contrast, DC induced significant responses of yo T cells at lower doses of stimulus, and the responses were even higher at optimal doses. ConA is known to mimic the cross-linking signal 1 provided by TCR interaction, resulting in the mitogenic stimulation of T cells independently of MHC-II antigen. Thus, the observed proliferation differences of yo T cells to ConA in the presence of monocytes or DC are rather due to the differences in the expression of CD80/CD86 molecules and not of the MHC-II antigens. By analogy, the pronounced expression of MHC-II on DC could be of lesser importance than the costimulatory molecules in the high
induction of yo T cells observed in the presence of SAgs. The better yo T-cell response to SAgs and ConA in the presence of DC, in comparison with monocytes, suggests that the sensitivity of yo T cells to stimuli is a function of the expression level of CD80/CD86 costimulatory molecules on APC. The monocytes are generally regarded as less efficient APC for the activation of naive T cells in vitro [28]. This is attributed to the low expression of MHC-II and costimulatory molecules. All in all, the important role attributed to macrophages in the initiation of the immune response in vivo would reside in their phagocytic capacity and the resulting cooperation with DC for the specific response of T cells against antigen.

![Diagram](image)

**Fig. 4.** Blocking of CD80 and CD86 on dendritic cell (DC) affects differently the WC1+ yo T-cell proliferation. WC1+ yo T cells (2 x 105/well) were cultured in triplicate with autologous y-irradiated DC (2 x 104 cells/well) with staphylococcal enterotoxin A (SEA) (A), staphylococcal enterotoxin B (SEB) (B), toxic shock syndrome type-1 (TSST-1) (C) at 103 ng/ml or concanavalin A (ConA) (D) at 10 μg/ml and interleukin (IL)-2 (50 U/ml) in the presence or absence of anti-CD80 and/or anti-CD86 antibodies at a final concentration of 1 μg/ml. Isotype-matched mouse immunoglobulin (IgM) and mouse IgG2b were used as controls. Proliferation was assessed by thymidine incorporation during the last 18 h of 4 days of culture. The results are expressed as counts per minute (cpm), and each point represents the mean ± standard deviation (SD) from three independent experiments. NS, not significant; *P<0.05; **P<0.01.

To further assess the role of CD80 and CD86 molecules, anti-CD80 and/or anti-CD86 antibodies were added as blocking agents. Our data demonstrate that WC1+ yo T-cell proliferation induced by SAgs and ConA in the presence of DC is dependent on the costimulatory signals. Indeed, while anti-CD86 alone inhibited the proliferation, the combination of both anti-CD80 and anti-CD86 suppressed the response. Moreover, the finding that both anti-CD80 and anti-CD86 MoAbs did not inhibit APC-independent bovine T-cell proliferation induced by PMA + ionomycin reagents strongly supports this conclusion. The possibility that MoAb anti-CD80 could cross-link human MHC-II molecules has been evoked. Our data show, however, that this antibody does
not influence the MHC-II/SAg presentation, as the addition of bovine anti-MHC-II but not anti-CD80 sup- presses SEA-mediated T-cell proliferation. Yokomizo et al. have shown that the bovine T-cell proliferation in response to SAg was MHC-II restricted. The addition of anti-CD86 MoAb, but not anti-CD80, in the cultures significantly inhibited the WC1+ yo T-cell proliferation, and the combination of both antibodies suppressed the response indicating the dominant role of CD86 in yo T-cell activation. In vitro studies suggest that the CD86 molecule on professional APC provides an initial signal to induce the proliferation of T cells, whereas CD80 interacting with CD28 provided a neutral differentiation signal. The results of Lienersley et al. showing that CD80 was a low-affinity ligand for CD28 extended this hypothesis. In the studies of other roles of the costimulatory molecules, several reports in vivo and in vitro suggested that the engagement of CD86 provided an initial signal to induce naive T cells to become T-helper cells (Th2), whereas CD80 was believed to drive a neutral signal. In conclusion, our data demonstrate that: (i) DC and monocytes presenting the SAg stimulate the proliferation of WC1+ yo T cells; (ii) this response requires exogenous IL-2 in both instances; (iii) DCs were more efficient than monocytes in inducing yo T-cell proliferation; and (iv) the costimulation delivered by the CD86 molecule or by the combination of CD80 and CD86 molecules expressed on APC was of primary significance for the activation of resting yo T cells.

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