Glucocorticoids Regulate TCR-Induced Elevation of CD4: Functional Implications

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Glucocorticoids Regulate TCR-Induced Elevation of CD4: Functional Implications

G. Jan Wiegars, Ilona E. M. Stec, Wolfgang E. F. Klinkert, and Johannes M. H. M. Reul

CD4 serves as a coreceptor during Ag recognition by the TCR. This interaction results in a marked increase in the sensitivity of a T cell to Ag presented by MHC class II molecules. Here we report that activation of T cells either by plate-bound mAb (anti-TCR, anti-CD3) or soluble activators (staphylococcal enterotoxin A, Con A) is associated with an (up to 3-fold) increase in CD4 cell surface expression on CD25+ cells, which was maximal after 72–96 h. Incubation with the glucocorticoid hormone cortisol (CORT) shifted the enhancement of CD4 expression to a point about 24 h earlier than that observed in control cultures. In parallel, the proliferative response of these CORT-treated cells was profoundly enhanced. An involvement of increased CD4 expression in this enhanced proliferative response was evidenced by the observation that T cell proliferation in CORT-treated cultures was much less sensitive to inhibition by an inhibitory, nondepleting anti-CD4 mAb than that in control cultures. TCR down-regulation was, however, not affected by CORT. Thus, based on this study and previous reports we propose that both TCR-mediated signals and glucocorticoids are important physiological regulators of CD4 expression. In addition, these findings may be of significance for the sensitivity of CD4+ cells to HIV infection upon T cell activation, as the efficacy of primary patient HIV entry depends on the level of surface CD4.

C

D4 is a transmembrane glycoprotein of 55 kDa belonging to the Ig gene superfamily (1). By binding to MHC class II molecules, CD4 serves as a coreceptor during Ag recognition by the TCR. This results in a marked increase in the sensitivity of a T cell to Ag presented by MHC class II, effectively lowering the dose of Ag required for activation by about 100-fold (2). To realize this function it has recently been proposed that CD4, by its ability to oligomerize, contributes to the formation of a cooperative assembly of TCR-MHC class II complexes (3–5). During T cell activation, CD4 and triggered TCRs are down-regulated and degraded with identical kinetics (6, 7). This process is the consequence of binding of the CD4-associated Src family tyrosine kinase Lck to ZAP-70 (7). Phosphorylation of ZAP-70 by Lck (or other kinases) induces the activation of different signaling pathways, ultimately leading to cell cycle progression, cytokine synthesis, or activation-induced cell death (8–10).

Several other important roles for CD4 have been documented. During in vivo thymocyte development, the CD4-MHC class II interaction is critical for the differentiation of CD4+CD8+ double-positive thymocytes into CD4+CD8− single-positive cells (11). In addition, CD4 has been reported to act as a receptor for IL-16, which induces chemotaxis of CD4+ cells and is a competence growth factor for CD4+ cells (12). Interestingly, recent evidence indicates a direct role for CD4 in apoptosis (13) and neurodegeneration (14), demonstrating that the functional properties of CD4 apparently are remarkably multifaceted. Finally, besides its physiological functions, CD4 acts as the primary receptor for HIV, enabling its entry into the immune system (15–17). Although the functional effects of CD4 as a participant in T cell activation are well documented, very little is known about its regulation. As a direct result of TCR triggering, CD4 surface expression is rapidly (within minutes) down-regulated (6, 7, 18, 19), and normalization of CD4 expression does not occur within 48 h (19). Expression of its counterpart, CD8, is down-regulated with the same kinetics as CD4 (7). In contrast, induction of de novo CD8 expression has been reported to occur upon activation in a population of CD4+ T cells by 72–120 h (20–22), a process that is potently stimulated by glucocorticoid hormones (23). In the thymus, signaling via the pre-TCR complex in CD4−CD8+ thymocytes leads to the induction of CD4 and CD8 expression and development into CD4+CD8+ cells (24). Interestingly, the conversion of CD4−CD8+ to CD4+CD8+ thymocytes is also greatly enhanced by glucocorticoids (25). These permissive actions of glucocorticoids are in contrast to their well-known inhibitory effects on various immune and inflammatory responses. It is generally assumed that the inhibition of the production of cytokines by glucocorticoid hormones accounts for the suppressive effects of these hormones (26). However, a rather paradoxical picture emerges as glucocorticoids up-regulate the expression of cytokine receptors. To date, it has been shown that receptors for IL-1, IL-2, IL-4, IL-6, IFN-γ, GM-CSF, CSF-1, as well as the common signal transducer gp130 are induced by glucocorticoids on several cell types (for review, see Ref. 27). Although, in general, the functional relevance of these contradictory effects of glucocorticoids is not known, it has recently been proposed that the net effect of restricting the synthesis of a given cytokine and simultaneously inducing its receptor may lead to a faster development of the biological response, which is thereafter rapidly terminated (27).

In our studies we investigated the role of glucocorticoids in T cell activation, especially focussing on their effects on membrane receptor expression. Here we show that activation of T cells either by plate-bound mAb (anti-TCR, anti-CD3) or soluble activators...
(staphylococcal enterotoxin A (SEA), Con A) is associated with a gradual (up to 3-fold) increase in CD4 membrane expression. Incubation with the glucocorticoid hormone corticosterone (CORT) profoundly accelerated the enhancement of CD4 expression. Importantly, the different levels of surface CD4 expression between control and CORT-treated cells appeared to be of functional significance for T cell activation, as evidenced by inhibition studies with anti-CD4 mAb. In contrast, activation-induced TCR down-regulation was not affected by CORT. Thus, CD4 expression is increased upon TCR triggering and is regulated by physiologic concentrations of glucocorticoid hormones. Moreover, these data put forward the testable hypothesis that T cell activation facilitates entry of HIV into CD4+ cells by increasing membrane CD4 expression.

Materials and Methods

Animals

Male Wistar rats (Charles River Wiga, Sulzfeld, Germany), weighing 200–250 g, were used for all experiments. They were housed under standard light (lights on from 0600–2000 h) and temperature (23°C) conditions. Food and tap water were available ad libitum. The experimental protocols were approved by the ethical committee on animal care and use of the government of Bavaria, Germany.

Abs and reagents

Purified mouse mAb reactive with rat TCRab (clone R.73) (28), mouse anti-rat CD3 (clone 1F4; Serotec, Kidlington, U.K.), Con A (Pharmacia, Uppsala, Sweden), and SEA (St. Louis, MO) were used for T cell mitogenic stimulation. For flow cytometry, FITC-conjugated mouse anti-rat TCRab IgG1 mAb, FITC-conjugated mouse anti-rat CD4 IgG1 mAb (clone W3/25, which recognizes an epitope of domain I of CD4), FITC-conjugated mouse anti-CD8a IgG1 mAb (clone OX8), PE-conjugated anti-CD4 mAb (W3/25), PE-conjugated anti-CD8a mAb (OX8), and PE-conjugated mouse anti-CD25 IgG1 mAb (clone OX39) were purchased from Serotec. FITC-conjugated mouse anti-rat CD4 IgG2a mAb (clone OX35, which binds to domain 2 of CD4) was obtained from PharMingen (San Diego, CA). Isotype control FITC-conjugated mouse IgG1 and IgG2a mAb, or PE-conjugated IgG1 mAb or PE-conjugated IgG2a mAb were obtained from Dianova (Hamburg, Germany). Purified mouse IgG1 mAb (Serotec) was used for functional CD4 inhibition studies. Purified mouse anti-rat CD43 (clone W3/13) served as an isotype control. CORT was obtained from Sigma. Murine rIL-2 (sp. act., 1.1 × 10^7 units/mg protein) was obtained from Becton Dickinson (Bedford, MA). RU486 (17-hydroxy-11-(4-dimethylaminophenyl)-17-(1-propynyl)ester-4,9-diene-3-one) was provided by Rousset-UCLAFL (Romainville, France). [methyl-3H]Tdr (sp. act., 2 Ci/mmol) was obtained from Amersham (Braunschweig, Germany).

Cell preparations and cultures

Spleens were removed aseptically between 0900–1000 h and gently disrupted through a screen cloth (pore size, 40 μm) to obtain single-cell suspensions. Cells were then centrifuged (10 min, 400 × g), and the pellet was resuspended in lysis buffer (155 mM NH₄Cl, 10 mM KHCO₃, and 0.1 mM EDTA) and maintained on ice for 6 min to lyse erythrocytes. Cells were then centrifuged (10 min, 400 × g) to obtain single-cell suspensions. Cells were activated with Con A (0.5–4.5 μg/ml). The soluble stimulants SEA (1 μg/ml) and Con A (1.5 μg/ml) were added to the cultures. Cells were cultured for 24–120 h and analyzed by double-immunofluorescence staining with PE-conjugated anti-CD25 mAb and FITC-conjugated anti-CD4 mAb. Unrelated PE- or FITC-conjugated IgG1 mAb were used as controls. Results (representative of four to six independent experiments) are expressed as the mean fluorescence intensity (MFI). Mean CD4 fluorescence of freshly isolated cells was 580 ± 9 for mAb control. Similar results were obtained after staining with Ox35 (data not shown). B, FACS plot of SEA-activated CD4+ T cells (as shown in A) after 24 h (solid lines) or 96 h (dashed lines) days of incubation. The histograms on the left show control staining with unrelated FITC-conjugated IgG1 mAb. C, CD4 expression by CD25- vs CD25+ , SEA-treated CD4+ T cells (as described in A) after 24–72 h of culture.

FIGURE 1. CD4 expression increases after T cell activation independently of the stimulus used to activate CD4+ T cells. A, Cells were activated with either plate-bound anti-TCR or anti-CD3 mAb (1 μg/ml) or the soluble stimulants SEA (1 μg/ml) and Con A (1.5 μg/ml). Cells were cultured for 24–120 h and analyzed by double-immunofluorescence staining with PE-conjugated anti-CD25 mAb and FITC-conjugated anti-CD4 mAb. Unrelated PE- or FITC-conjugated IgG1 mAb were used as controls. Results (representative of four to six independent experiments) are expressed as the mean fluorescence intensity (MFI). Mean CD4 fluorescence of freshly isolated cells was 580 ± 9 for mAb control. Similar results were obtained after staining with Ox35 (data not shown). B, FACS plot of SEA-activated CD4+ T cells (as shown in A) after 24 h (solid lines) or 96 h (dashed lines) days of incubation. The histograms on the left show control staining with unrelated FITC-conjugated IgG1 mAb. C, CD4 expression by CD25- vs CD25+ , SEA-treated CD4+ T cells (as described in A) after 24–72 h of culture.
CD4 inhibition studies

A mouse anti-rat CD4 mAb (W3/25), known to inhibit CD4+ T cell activation without depleting these cells (29), was used in our experiments. Cells were incubated in the absence or the presence (10^{-7} - 10^{-6} M) of CORT with various concentrations of W3/25 mAb (0.005–5 μg/ml) or an isotype control mAb. Anti-TCRα- or anti-CD3-induced T cell proliferation was assessed after 48 h of culture as described above. Saturation of surface CD4 binding sites by incubation with W3/25 (5 μg/ml) was checked for by the determination of (residual) staining with FITC-conjugated W3/25 mAb.

Flow cytometry

Cells were washed twice with PBS containing 0.01% NaN3 and 1% BSA. Cell surface expression of CD4 or CD8α was examined by (double) staining with FITC-conjugated anti-CD4 mAb (W3/25 or OX35, which bind to different domains of CD4 (domains 1 and 2, respectively) and do not compete for binding (30) or with FITC-conjugated anti-CD8α mAb and PE-conjugated mouse anti-rat CD25 mAb. Activation-induced modulation of TCRαβ on CD4+ cells was measured with FITC-conjugated anti-TCRαβ and PE-conjugated mouse anti-CD25 mAb. In some experiments double staining with FITC-conjugated anti-CD4 mAb and PE-conjugated anti-CD8α mAb was performed. Unrelated FITC-conjugated IgG1 and IgG2a mAb or PE-conjugated IgG1 mAb were used as controls. Staining continued for 30 min on ice in the presence of 10% normal rat serum. Cells were washed twice with PBS supplemented as described above and analyzed on a FACSort (Becton Dickinson, Sunnyvale, CA). To adequately determine mean fluorescence levels of CD4, CD8α, and TCRαβ over time or after treatment with CORT, instrument settings were kept constant throughout the experiments, and all stainings were performed with the same batch of each mAb.

Results

CD4 expression increases after T cell activation

Several T cell surface proteins are modulated upon TCR triggering, resulting in either de novo expression (e.g., CD25) (31), up-regulation of constitutively expressed proteins (e.g., CD2 (32) and CD27 (33)), or down-regulation (TCR/CD3 complex) (34–36). The expression of CD4 and its mRNA after TCR triggering have been extensively studied (6, 7, 18, 19, 37, 38). Activation of T cells

Table I. Effect of CORT on CD4 expression after T cell activation

<table>
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<tr>
<th>Stimulus</th>
<th>CORT (5 × 10^{-7} M)</th>
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<th>48 h</th>
<th>72 h</th>
<th>96 h</th>
<th>120 h</th>
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<td>501</td>
<td>1103</td>
<td>1508</td>
<td>1285</td>
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<tr>
<td></td>
<td>+</td>
<td>519</td>
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<tr>
<td>Anti-CD3</td>
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<td>509</td>
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<td>916</td>
<td>1055</td>
<td>1068</td>
<td>1024</td>
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<tr>
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<td>426</td>
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<td>442</td>
<td>980</td>
<td>1017</td>
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*CD25+ cells were analyzed for expression of CD4 with a FITC-conjugated W3/25 mAb at the indicated times. Results (representative of four to six independent experiments) are expressed as MFI. The MFI of fresh cells was 584 vs 8 for mAb control.

CD25+ cells were analyzed for expression of CD4 with a FITC-conjugated W3/25 mAb at the indicated times. Results (representative of four to six independent experiments) are expressed as MFI. The MFI of fresh cells was 584 vs 8 for mAb control.

FIGURE 2. CORT accelerates the rise in CD4 expression after T cell activation. Cells were incubated in the absence or the presence of CORT (10^{-6} M) and were simultaneously activated with plate-bound anti-TCR (1 μg/well). Cells were cultured for 24–120 h and analyzed as described in Fig. 1. Results (representative of six independent experiments) are expressed as the MFI of CD4. Similar results were obtained after staining with OX35 (data not shown). B, FACS plot of anti-TCR-activated CD4+ T cells (as shown in A) in the absence (solid lines) or the presence (dashed lines) of CORT after 48 h of incubation. The histograms on the left show control staining with unrelated FITC-conjugated IgG1 mAb.

FIGURE 3. The effects of CORT on CD4 expression are reversed by RU486. Cells were preincubated with the glucocorticoid receptor antagonist RU486 (5 × 10^{-7} M) or medium control 30 min before activation with anti-TCR mAb (1 μg/well) and addition of either medium or CORT (5 × 10^{-7} M). Cells were cultured for 48 h and analyzed as described in Fig. 1. Results (representative of three independent experiments) are expressed as the MFI of CD4.
A previous report demonstrated that glucocorticoids induce CD8 cells, indicating that activation is a prerequisite for CD4 expression by Ag has been shown to result in a 15–50% decrease in membrane CD4 expression (7, 18, 19) after 6–48 h. No substantial change in CD4 mRNA expression has been found after CD3 triggering (38). Most of these studies investigated CD4 expression during a time window ranging from several hours to 48 h. We studied the expression of CD4 on rat splenic CD25+ cells after 24–120 h. Cells were activated with either plate-bound anti-TCR or anti-CD3 mAb or the soluble stimulants SEA and Con A. CD4 expression (as analyzed by flow cytometry) was compared with that in fresh, unstimulated cells. Independently of the stimulus used to activate CD4+ T cells, a decrease in surface CD4 expression was observed, ranging from 10–40% by 48 h of culture (Fig. 1A and Table I). Surprisingly, independently of the stimulus, TCR triggering induced a potent increase in CD4 levels (Fig. 1 and Table I), reaching a maximal 2- to 3-fold induction by 96 h. The idea that SEA only activates a subset of T cells enabled us to study CD4 expression on unactivated CD25− cells. Fig. 1C shows that, in contrast to CD25+ cells, CD4 expression was not induced on CD25− cells, indicating that activation is a prerequisite for CD4 induction.

**CORT accelerates the rise in CD4 expression after T cell activation**

A previous report demonstrated that glucocorticoids induce CD8 α-chains on Con A- and Ag-activated CD4+ T cells (23). The increased expression of CD4 after T cell activation as observed in our experiments prompted us to investigate whether this process may be regulated by glucocorticoids. Splenic lymphocytes were incubated in the absence or the presence of CORT (10−6 M) and were simultaneously activated either with plate-bound mAb against TCR or CD3 or with soluble stimulants (Con A, SEA). After 48 h of culture, CORT induced a marked increase in CD4 expression (up to 2.3-fold), followed by a moderate inhibition after 96–120 h of culture compared with that in untreated controls (Fig. 2 and Table I). The effects of CORT on CD4 expression were dose dependent (data not shown).

**The effects of CORT on CD4 expression are reversed by RU486**

Both the inhibitory and stimulatory effects of CORT on T cell mitogenesis have been shown to be reversed by the glucocorticoid receptor antagonist RU486 (39, 40). Next, we tested whether the effects of CORT on CD4 expression were mediated by the glucocorticoid receptor. Splenic lymphocytes were preincubated with 5 × 10−6 M RU486 30 min before activation with anti-TCR mAb and addition of CORT (5 × 10−7 M). The CORT-evoked increase in CD4 expression after 48 h of culture was completely abolished by RU486 (Fig. 3), demonstrating that the effect on CD4 expression is glucocorticoid receptor mediated.

**CORT-evoked acceleration of CD4 induction is paralleled by increased CD4+ T cell proliferation**

It has been previously shown that CORT accelerates T cell proliferation in parallel with an increased IL-2Rα expression (40). Because CORT enhances the activation and proliferation that follow TCR engagement, the CORT-evoked acceleration of CD4 after 48 h may participate in the enhancement of T cell activation by this hormone. Indeed, the enhanced expression of CD4 after 48-h culture in the presence of CORT was paralleled by an increased anti-TCR-induced proliferative response (as assessed by [3H]Tdr incorporation) after 48–96 h (data not shown). In the presence of CORT, the number of CD4+CD25+ cells was increased 2- to 3-fold by 72–96 h (Fig. 4A). Similar results were obtained after activation with SEA (Fig. 4B).

**Anti-CD4 mAb differentially affects CD4+ T cell proliferation in the presence or the absence of CORT**

To directly address the functional significance of the CORT-accelerated induction of CD4, we tested whether a differential sensitivity to inhibition of the proliferative response by anti-CD4 mAb was present between control and CORT-treated cultures. Blocking CD4 by mAb has been reported to inhibit MLR (29) and Ag specific T cell activation (41). A nondepleting mAb to rat CD4 (W3/25) known to inhibit T cell activation (29) and Ag specific T cell activation (41). A nondepleting mAb to rat CD4 (W3/25) known to inhibit T cell activation (29) was tested on anti-TCR-specific T cell activation (41). A nondepleting mAb to rat CD4 (W3/25) known to inhibit T cell activation (29) was tested on anti-TCR-induced proliferation in the presence or the absence of CORT. W3/25 mAb dose-dependently inhibited T cell proliferation (Fig. 5; the highest concentration of W3/25 (5 μg/ml) was saturating, as no residual CD4 binding sites could be detected by staining with FITC-conjugated W3/25). However, in cultures incubated with CORT for 48 h (i.e., a point at which the expression of CD4 by these cells is increased at least 2-fold), much higher concentrations of this mAb were required to inhibit T cell proliferation (Fig. 5, A and B). Similar results were obtained after incubation with CORT for 72 h (Fig. 5, C and D). Incubation of cultures with an isotype control mAb did not significantly affect T cell proliferation (data not shown). Thus, increased CD4 expression in the presence of CORT was mirrored by a reduced sensitivity to inhibition by anti-CD4 mAb.
Activation-induced TCR down-regulation is not affected by CORT

TCR triggering is rapidly followed by down-regulation of TCRs (34, 42). Whether activated by Ag, superantigen, or anti-CD3 mAb, T cells become fully activated only if a certain threshold number of TCRs is down-regulated (43, 44). The finding that CORT induces an acceleration of CD4 expression and T cell proliferation prompted us to investigate whether CORT would also modulate TCR down-regulation. A marked reduction of TCR expression (up to 85%) was observed 24–48 h after activation with SEA, anti-TCR mAb, anti-CD3 mAb, or Con A (Fig. 6A), followed by a gradual re-expression over the next 72 h. These results are in agreement with previous studies showing that TCR expression is maximally reduced after several hours, remains low for at least 24 h, and is gradually re-expressed thereafter (34, 43). In contrast to the stimulatory effects of CORT on CD4 expression, incubation of anti-TCR-activated cultures with the glucocorticoid hormone did not modify TCR down-regulation over the entire culture period of 120 h (Fig. 6B). Similar results were obtained after activation with SEA (data not shown).

**FIGURE 5.** Anti-CD4 mAb differentially affects CD4+ T cell proliferation in the presence or the absence of CORT. Cells were simultaneously incubated with different concentrations of anti-CD4 (W3/25; 0–5 μg/ml) mAb and plate-bound anti-TCR mAb (1 μg/well) in the absence (□) or the presence (■) of CORT (10^{-6} M). After 48 h (A and B) or 72 h (C and D) of incubation, cultures were pulsed with [3H]thymidine for 6 h before harvesting. In B and D, the results shown in A and C are expressed as a percentage of the control value, respectively. Results (expressed as mean ± SEM) are based on four independent experiments.

**FIGURE 6.** Activation-induced TCR down-regulation is not affected by CORT. A. Cells were activated as described in Fig. 1 and cultured for 24–120 h. TCR expression was analyzed by double-immunofluorescence staining with PE-conjugated anti-CD4 mAb and FITC-conjugated anti-TCR mAb. Unrelated PE- or FITC-conjugated IgG1 mAb were used as controls. Results (representative of four independent experiments) are expressed as the percent MFI of TCR expression by fresh cells (=100%; MFI = 389.8; MFI for mAb control, 8). B. Cells were incubated in the absence or the presence of CORT (10^{-6} M) and were simultaneously activated with plate-bound anti-TCR mAb (1 μg/well). Cell culture and TCR analysis were performed as described in A.

*Activation-induced TCR down-regulation is not affected by CORT*

TCR triggering is rapidly followed by down-regulation of TCRs (34, 42). Whether activated by Ag, superantigen, or anti-CD3 mAb, T cells become fully activated only if a certain threshold number of TCRs is down-regulated (43, 44). The finding that CORT induces an acceleration of CD4 expression and T cell proliferation prompted us to investigate whether CORT would also modulate TCR down-regulation. A marked reduction of TCR expression (up to 85%) was observed 24–48 h after activation with SEA, anti-TCR mAb, anti-CD3 mAb, or Con A (Fig. 6A), followed by a gradual re-expression over the next 72 h. These results are in agreement with previous studies showing that TCR expression is maximally reduced after several hours, remains low for at least 24 h, and is gradually re-expressed thereafter (34, 43). In contrast to the stimulatory effects of CORT on CD4 expression, incubation of anti-TCR-activated cultures with the glucocorticoid hormone did not modify TCR down-regulation over the entire culture period of 120 h (Fig. 6B). Similar results were obtained after activation with SEA (data not shown).
Discussion

In this study we have demonstrated that cell surface CD4 expression is increased upon activation of T cells by superantigen SEA, mAb against TCR and CD3, or Con A. The enhanced expression of CD4 is accelerated in the presence of glucocorticoids, which was paralleled by both an enhanced proliferative response of these cells and a reduced sensitivity of this response to inhibition by anti-CD4 mAb. Furthermore, glucocorticoid treatment did not affect TCR down-regulation. As glucocorticoids, on the one hand, induce CD4 and, as previously shown, CD8 expression on activated mature T cells (23) and, on the other hand, appear to be essential for the conversion of immature CD4+CD8- thymocytes to the CD4+CD8+ stage (25), we postulate that these hormones are important physiological regulators of CD4 and CD8 coreceptor expression. Finally, the increased expression of cell surface CD4 after T cell activation may lead to a facilitated entry of HIV into these cells, which would, in addition to the activation-induced transcriptional machinery, represent a new mechanism explaining the capacity of HIV to infect and replicate more effectively in activated vs resting T cells.

The regulation of CD4 expression on mature T cells is not well understood, and knowledge has been gained mainly on the down-regulation of cell surface CD4. The phorbol ester PMA potently induces CD4 down-regulation, which is detectable within 5 min after the onset of treatment and an almost complete loss of CD4 expression is observed after ~4 h. Re-expression of CD4, although at reduced levels, is seen after 24–48 h (18, 37, 45–49). Down-regulation of CD4 after TCR triggering is much less pronounced than that after PMA (6, 7, 18, 19, 37). Antigenic activation of T cells resulted in a 15–50% decrease in membrane CD4 expression (7, 18, 19) after 6–48 h, whereas stimulation by anti-CD3 mAb induced either a reduction (7, 19) or no change (37) in CD4 membrane expression. Our results on CD4 down-regulation (ranging from 10–40% after 48 h compared with freshly isolated cells) are in line with these studies.

Our data demonstrate an increased expression of CD4 72–120 h after TCR triggering. Recently, it has been shown that upon immunization and in vitro antigenic restimulation, Ag-specific T cells up-regulate CD4 expression by 72–96 h (50), which is consistent with our observation. Whereas most other previous studies investigated CD4 expression during a time window ranging from several hours to 48 h, we studied the expression of CD4 on CD25+ cells after 24–120 h. Evidence for a functional role of CD4 up-regulation in T cell activation is provided by our observation that an increased CD4 expression in the presence of glucocorticoids was mirrored by a reduced capacity of anti-CD4 mAb to inhibit T cell activation. Moreover, the observation that the glucocorticoid-accelerated CD4 up-regulation is paralleled by an increased cellular proliferation suggests that, at least under our experimental conditions, glucocorticoids act to optimize CD4 expression and the proliferative response. Germain and co-workers proposed that many T cells may operate at the limit of the system, i.e., even a modest decrease in coreceptor availability is sufficient to change a stimulatory agonist into a partial agonist (51). Thus, it is becoming increasingly clear that the number of (activatory) cell surface molecules per cell is an essential parameter for the magnitude of the overall biological response of a given cell. The level of cell surface CD4 directly correlates with its capacity to function as a chemotaxiant for IL-16 (12). Positive and negative selection of T cells is directly influenced by the level of either CD4 or CD8 expression (52–61). The quantity of activation-induced IL-2Rα (CD25) per cell determines the cell’s capacity to rapidly progress through the cell cycle (31). In addition, T cells may down-regulate up to 90% of their TCR within a period of 10 h after TCR triggering. However, this is not sufficient to commit these cells to proliferation, as this process appears to take 15–20 h in naive T cells (62). The supply of newly synthesized TCR at the cell surface may be required to sustain triggering of TCR to achieve full commitment (62). Whether glucocorticoids may increase the turnover of TCR was not addressed in our study. However, as TCR down-regulation as well as re-expression were not affected over time by glucocorticoids, we presently favor the hypothesis that, by inducing CD4, these hormones may quantitatively and/or qualitatively modify the signal delivered at TCR triggering. In line with current models of TCR signaling, the glucocorticoid-induced increase in CD4 expression may lead to a higher number of productive TCR-ligand interactions (1, 4), thereby speeding up the process of T cell activation. Nonetheless, the induction of CD4 by glucocorticoids is not the only mechanism by which these hormones augment proliferation, as saturating concentrations of inhibitory anti-CD4 mAb in the presence of CORT did not completely reduce the proliferative response to control levels. Accordingly, among other mediators, the (direct or indirect) induction of CD25 by CORT (40) probably plays, in concert with enhanced CD4 expression, an important role in the CORT-evoked enhancement of T cell proliferation.

Several studies have shown that T cell activation induces CD8 on CD4+ cells, thereby generating a CD4+CD8− subset, the function of which is not known (20–22). Our results are in agreement with those of Ramirez (23) in that, on the one hand, T cell activation by Con A generates a population of double-positive CD4+CD8+ cells and, on the other hand, glucocorticoids greatly increase both the frequency of these cells and the mean expression of CD8 (G. J. Wiegers et al., unpublished observation; similar results were obtained after activation with anti-TCR mAb (data not shown)). However, the study of Ramirez et al. did not report any effects of T cell activation or glucocorticoid hormones on CD4. This may be due to the circumstance that an increase in CD4 may have escaped detection, as Ramirez et al. measured CD4 and CD8 expression only at 72 h. Our data show that Con A-activated T cells by this time expressed only moderately increased levels of CD4 (~40%), and maximal levels were reached at 96–120 h of culture, whereas activation with SEA, anti-CD3, or anti-TCR induced a near-maximal increase in CD4 levels by 72 h (~100%). In the presence of glucocorticoids, maximal CD4 induction compared with control cells is noted 48 h after T cell activation and is much less pronounced by 72 h. Thus, T cell triggering induces the expression of both CD4 and CD8 on mature T cells, and glucocorticoids accelerate this process. Interestingly, immature CD4−CD8− thymocytes start to up-regulate CD4 and CD8 after an immature form of the TCR (i.e., consisting of a pre-TCR α-chain, covalently associated with a rearranged TCR β-chain) has been expressed (63). This pre-TCR, in association with CD3, transduces signals in CD4+CD8+ thymocytes. Moreover, expression of the pre-TCR on CD4+CD8− cells is required for the progression to the CD4+CD8+ stage (24, 64). A striking analogy exists between the regulation of CD4 and CD8 expression by glucocorticoids in mature T cells vs immature thymocytes. Transgenic mice expressing antisense transcripts to the glucocorticoid receptor selectively in immature thymocytes display severely disturbed thymocyte maturation (25). The hyporesponsiveness of thymocytes to glucocorticoids results in a reduction of the number of thymocytes by 90%, primarily due to a decrease in the CD4+CD8− subset. Consistent with this observation, glucocorticoids were shown to be important during the proliferative phase that accompanies the transition from CD4−CD8− to CD4+CD8+ cells (25). Thus, both TCR triggering and glucocorticoids are required for the transition...
from immature CD4+ CD8− to CD4+ CD8+ cells. Taken together, we propose that TCR-mediated signals as well as glucocorticoids are important physiological regulators of CD4+ and CD8+ coreceptor expression in both immature and mature T cells.

Thymus-derived glucocorticoids have been shown to regulate Ag-specific positive selection. Hence, inhibition of thymic glucocorticoid synthesis evokes apoptosis of CD4+ CD8+ cells expressing a transgenic TCR that would otherwise have undergone positive selection (65). The molecular basis of this glucocorticoid effect is not known. In view of the observation that these hormones induce CD4+ and CD8+ molecules on mature T cells, it is tempting to speculate that a similar induction of these coreceptors on immature thymocytes may be instrumental for the regulation of positive selection.

The induction of CD4+ is probably important for a number of its physiological roles in the immune system such as T cell activation (see above), IL-16-induced chemotaxis (12), and even, as has been recently shown, apoptosis (13) and neurodegeneration (14). However, increased CD4+ expression may also be of direct immunopathological significance, particularly with regard to HIV pathogenesis. It is known that infection of CD4+ cells by T cell tropic HIV is a very inefficient process (66). Interestingly, primary patient, but not laboratory-adapted, T cell-tropic HIV-1 isolates infect in proportion to the level of cell surface CD4 (67, 68). This is probably due to the lower affinity of primary patient HIV-1 isolates for binding to CD4 than their laboratory-adapted derivatives, apparently leading to interactions of limited productivity with the molecule (66–69). In addition, evidence exists that HIV infection of CD4+ cells may require diffusion of additional CD4 molecules into the site of attachment (70–72), thereby reducing virus dissociation and facilitating the membrane reaction. Based on these observations, our experiments suggest that T cell activation may render the cell susceptible for HIV entry due to an increased expression of cell surface CD4. Indeed, previous studies have shown that activation of T cells strongly contributes to HIV replication (73–77). Up to now, however, these findings have been explained by an interplay of viral and host regulatory proteins, such as Tat, Rev, Nef, NF-κB, and NF-AT. The interaction between these viral and cellular proteins leads to increased HIV long terminal repeat-driven transcription and viral replication (78, 79).

We propose that T cell activation may influence HIV infection in two ways. First, an activation-induced increase in cell surface CD4 expression facilitates HIV entry into uninfected cells. This process may be promoted by glucocorticoid hormones. Second, the activated transcriptional machinery promotes HIV replication in these cells.

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References


