Glucocorticoids Induce the Expression of CD8α Chains on Concanavalin A–activated Rat CD4+ T Cells: Induction Is Inhibited by Rat Recombinant Interleukin 4

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Summary

Rat T lymphocytes, activated in vitro with concanavalin A (Con A), were shown by flow cytofluorographic analysis to contain a population of cells that simultaneously expressed CD4 and the α chain of CD8. The inclusion of the glucocorticoid hormone dexamethasone in the culture medium greatly increased both the frequency of these double-positive cells and the level of CD8α chain expression. The level of expression of CD4 was not affected, and the cells that expressed CD8 antigen only also remained unchanged in surface phenotype. Detailed studies demonstrated unequivocally that the CD4+CD8α− cells were not artifacts produced by the random association of single-positive cells in the flow cytofluorograph, but arose from precursors that were single-positive CD4+ cells before activation. Furthermore, Con A activation of purified CD4+ T cells, in the presence of T cell–depleted accessory cells, showed that CD8+ T cells played no role in the induction process. However, the induction of CD8α chain expression on CD4+ T cells and the enhancement of this expression by dexamethasone were almost completely inhibited by rat recombinant interleukin 4 (IL-4). Detection of mRNA for rat CD8α chain by Northern blot closely paralleled the cell surface expression of CD8α antigen, indicating that dexamethasone and IL-4 had opposing effects on mRNA levels. In contrast, IL-4 and dexamethasone both induced CD8α chain expression on a rat CD4+ T cell clone when this was activated by specific antigen, and, although the effect with IL-4 was relatively weak, it did not antagonize the effect of the glucocorticoid. The possible significance of these results is briefly discussed.

Glucocorticoids are widely used as immunosuppressive and antiinflammatory agents. Their immunosuppressive effects, although not well understood, probably depend in part on their property of depressing the expression of many interleukin genes (1–3). However, not all the effects of glucocorticoids are inhibitory. It has recently been reported that they enhance the synthesis of IgE when added to cultures of B cells containing IL-4 (4) and induce the expression of several cytokine receptors on various mononuclear cells (5–8). These observations suggest that the overall effect of these hormones will depend on both positive and negative actions.

Cell surface expression of CD4 and CD8 molecules divides mature T cells into two functionally different subsets. The majority of normal peripheral T lymphocytes express either CD4 or CD8 antigens, although a small percentage of circulating lymphocytes coexpress CD4 and CD8 (9). This circulating double-positive population consists of lymphoblasts, suggesting that CD4 and CD8 coexpression on mature T cells is related to activation. In accordance with this observation, it has been shown in humans (9, 10) and rats (11) that a CD4+CD8+ population is generated after activation of T lymphocytes by mitogen or MLR. The generation in vivo of a double-positive population has been reported in the rat after activation in several ways: in the spleen of rats primed with dinitrophenyl bovine gamma globulin (12), in the blood of cyclosporine-treated heart-allografted rats (13), in the peritoneal exudate from rats immune to a syngeneic rat mammary adenocarcinoma (14), and in the thoracic duct lymph of animals undergoing GVHD (D. Mason, unpublished results). In humans there are similar findings: the existence of a double-positive population has been described in patients with rheumatoid arthritis (15) and after renal transplantation (16). These reports suggest that after activation, in vivo or in vitro, some T cells develop a transient stage characterized by CD4 and CD8 coexpression, an unusual phenotype in mature T cells. It should be pointed out, however...
ever, that not all groups have found CD4+CD8+ double-positive T cells after activation, and it has been suggested that the majority of these cells may be artifacts produced by association of single-positive cells during flow cytofluorographic analysis (17).

Our initial aim was to examine the action of glucocorticoids during T cell activation, specifically asking whether there is a different effect upon CD4+ and CD8+ T cells. Surprisingly, we observed a very extensive overlap in the percentages of CD4+ and CD8+ populations after mitogen activation in the presence of the glucocorticoid analogue dexamethasone, suggesting that a very large population expressing both cell surface molecules was being generated. Using two-color immunofluorescence analysis, we have demonstrated that CD4+ single-positive cells are the precursors of the double-positive ones. This latter population probably arises by de novo biosynthesis of CD8α, because we can detect CD8α-specific mRNA in T cells that, before activation, were virtually all CD4+CD8-. We also show the existence of hormonal and lymphokine regulation for the transient expression of CD8αc in CD4+ T cells, with glucocorticoids increasing, and IL-4 decreasing, the levels of expression. The possible in vivo relevance of these observations is discussed.

Materials and Methods

Animals. PVG.RT1, PVG.RT1+, and PVG.RT7+ strain rats were from the specific pathogen-free unit of the Medical Research Council Cellular Immunology Unit.

Antibodies. mAbs used in this work were as follows: W3/25 (anti-rat CD4) (18), MRC-OX35 (anti-rat CD4 noncompe titive with W3/25) (19), MRC-OX8 and MRC-OX9 (anti-rat CD8) (20), MRC-OX22 (anti-rat Ig κ chain) (21), MRC-OX6 (anti-rat MHC class II) (22), MRC-OX22 (anti-rat CD45-restricted determinant) (23), MRC-OX32 (anti-rat CD45-restricted determinant noncompe titive with OX22) (24), NDSS8 (anti-rat CD45 allo type RT7+) (25), HIS 41 (anti-rat CD45 allotype RT7+) (26), MRC-OX21 (anti-human C3b inactivator) (27), and mAb 341 (reactive with rat CD8 heterodimers but not with CD8αe chain) (28). Biotinylated mAbs were prepared as described (29).

Rat Recombinant Interleukins and Other Reagents. Tissue culture supernatants containing either rat rIL-2 or rat rIL-4 were obtained from stably transfected Chinese hamster ovary (CHO) cell lines, generated using the rat IL-2 (30) or rat IL-4 (31) cDNA inserts sub cloned into the pE6.HCMV-GS expression vector (32). Supernatants from mock-transfected CHO cells served as controls in each of the experiments. Rat rIFN-γ was the generous gift of Dr. P. van der Meide (Primate Centre TNO, Rijswijk, The Netherlands).

Dexamethasone (Sigma Chemical Co., St. Louis, MO) was stored as a stock solution at 10⁻⁵ M in ethanol, and was diluted into complete media just before its addition to culture.

Cells. Rat thoracic duct lymphocytes (TDL) were obtained by cannulation of the duct (33). Cells were collected at 4°C overnight into flasks containing PBS and 20 U/ml heparin. Spleens were removed aseptically from rats, and the cells were isolated by pressing fragments of the tissue through a stainless steel mesh into ice-cold PBS containing 0.2% BSA. Myelin basic protein (MBP) is a MBP-specific CD4+ T cell line; its production and maintenance have been previously described (34).

Isolation of Subsets of Lymphocytes. Subpopulations of TDL, lymph node cells, and splenocytes were purified by negative selection using the rosetting technique (29). T cells were purified by removing cells expressing OX12 and OX6 antigens. CD4+ T cells were isolated by depletion of the OX12+, OX6+, and OX8+ cells. CD8+ T cells were obtained using the mAbs W3/25, OX35, and OX6. T cell–depleted splenocytes, used as accessory cells, were prepared by depletion of W3/25-, OX35-, and OX8-positive cells. OX22hi, CD4+ and OX22lo, CD4+ subpopulations were obtained by cell fractionation using a FACS®II (Becton Dickinson & Co., Mountain View, CA), after labeling of isolated CD4+ cells with the mAbs OX22 and OX32, and FITC-conjugated rabbit anti mouse Ig. After all fractionation procedures, cell purities were assessed by labeling pre- and postdepletion samples with rabbit anti-mouse FITC and analyzing on a FACS®II flow cytofluorograph (Becton Dickinson & Co.). Purities were always 97–99%.

Cell Cultures. Cells were stimulated with 5 μg/ml Con A in RPMI containing 10⁻⁵ M 2-ME, 5% FCS, and antibiotics, in the presence of T cell–depleted splenocytes as accessory cells preirradiated with 2,000 rad ¹³⁷Cs γ irradiation. After a 3-d incubation period, cells were harvested and prepared for FACS® analysis or RNA isolation. The rIL-3 cell line was maintained by a cycle of 3 d of antigen stimulation (MBP at 20 μg/ml) in the presence of irradiated syngeneic thymocytes, followed by 10 d resting in the presence of rIL-2. Cells were separated over a Ficoll-Hypaque density gradient at the beginning of the activation period and before FACS® analysis.

Immunofluorescence Staining and Analysis. Viable lymphocytes, isolated at the end of the culture period, were incubated with 50 μl of tissue culture supernatant from the appropriate hybridoma culture, washed twice, and then incubated with fluoresceinated rabbit F(ab')2; anti-mouse IgG antibody in the presence of 10% normal rat serum to avoid anti-rat Ig crossreactivity. The cells were washed again and then analyzed on a FACS®II. The irrelevant mAb MRC OX21 was used as a control for specificity. To carry out two-color flow cytofluorography, the cells were incubated with the mouse mAb, washed, and then incubated with fluoresceinated Fab anti-mouse Ig antibody. After two further washes, the cells were incubated with the corresponding biotinylated mAb, followed, after two additional washes, by PE-conjugated streptavidin. At the end of this incubation period, unconjugated biotin, a final concentration of 3 μg/ml, was added to reduce cell aggregation after centrifugation. After 10 min of incubation with the biotin, the cells were given a final wash and analyzed on the FACS®II.

Northern Blot Analysis. Total cellular RNA was prepared by a guanidinium thiocyanate–pheno!-chloroform extraction technique (35), and 20 μg of each sample was subjected to electrophoresis through a denaturing 1.2% agarose gel. The RNA was transferred to a GeneScreen Plus membrane (New England Nuclear, Boston, MA) and probed with a 32P-labeled HindIII/EcoRI cDNA fragment encoding the carboxyl terminus of the rat CD8α chain (36). The membrane was stripped and reprobed with a 607-bp cDNA fragment derived from the rat β actin gene sequence (37) generated by using the PCR. After hybridization, membranes were washed with 1× SSC/1% SDS at 60°C and exposed to Hyperfilm-MP (Amersham International, Amersham, UK) at −70°C.

Results

Dexamethasone Potentiates the Generation of a CD4+CD8+ Population during Mitogen Activation. To study the effect of glucocorticoids on the expression of CD4 and CD8 on rat T cells, normal spleen cells were activated with Con A in
the absence or presence of dexamethasone. The inclusion of the hormone in the culture resulted in a marked increase in the number of cells expressing CD8, and an overlap in the percentages of CD4+ and CD8+ cells. To further investigate this phenomenon, purified T cells were activated with Con A using T cell–depleted splenocytes as feeder cells, in the presence of 10^{-6} or 10^{-8} M dexamethasone, considered pharmacological and physiological glucocorticoid concentrations, respectively. Two-color immunofluorescence analysis was carried out after a 3-d incubation period. Fig. 1 A shows the result of activating T cells with Con A alone.

The CD4+CD8+ double-positive population detected in this experiment amounted to 16% of the total cells analyzed. This percentage varied between different experiments, and sometimes the size of the double-positive population was markedly higher (see Fig. 3). Fig. 1 B and C, show that including dexamethasone in the culture increased the number of the CD4+CD8+ cells, and that this effect was dose dependent: 10^{-6} M induced a higher percentage (42%) than 10^{-8} M (27%). As in some previous reports (9, 11), it was observed that the CD4+CD8+ population, induced by a 3-d incubation of T cells in Con A alone, decreased with time if the Con A was removed and the cells were maintained in a proliferative state by the addition of IL-2. However, if the initial 3-d stimulation with Con A was made in the presence of dexamethasone and the subsequent culture was in IL-2 alone, then the frequency of double-positive cells actually increased significantly, suggesting that the level of steroid hormone present at the time of activation was important in determining the longer term behavior of the cells (data not shown).

**The CD4+CD8+ Population Derives from CD4+CD8-T Cells That Become Double Positive on Activation.** It has been argued that during T cell activation the true percentage of CD4+CD8+ cells is very low, and that doublets of single-positive cells make up the majority of the apparent double-positive population (17). To test this possibility, CD4+ and CD8+ cells were purified from two congenic rat strains expressing different allotypes of the leukocyte common antigen (LCA; CD45). The CD8+ cells were obtained from the strain PVG.RT1+ (RT7+ or LCA 1.1 allotype) and CD4+ cells from the strain PVG.RT7+ (RT7- or LCA 1.2 allotype). After purification, the CD8+RT7+ and CD4+RT7+ cells were mixed and activated with the mitogen.

Fig. 2 shows the phenotype of the cells after a 3-d incubation period in the presence of dexamethasone. The CD4+CD8+ population that was generated accounts for 45% of the cells in the culture (Fig. 2 A). Labeling with the mAbs NDSS8 and HIS 41 (Fig. 2 B) was used to investigate the possible
existence of doublets with a CD4⁺RT7ᵇ⁺/CD8⁺RT7ᵇ⁺ phenotype. This population amounted to only 2% of the total. It is evident that the few doublets of cells are not sufficient to account for the great majority of the 45% of cells coexpressing CD4 and CD8 antigens.

It was also determined in this experiment which T cell subset initially single-positive CD4 or CD8, becomes double positive. In Fig. 2 C, 43% of the cells express both RT7ᵇ⁻ and CD8 antigen after activation in the presence of dexamethasone. All of the RT7ᵇ⁻ cells express CD8, being the CD8⁺RT7ᵇ⁻ cells. Fig. 2 D shows the existence of two populations: CD4⁺RT7ᵇ⁻ and CD4⁺RT7ᵇ⁺. These results show that all of the RT7ᵇ⁻ cells, originally single-positive CD4⁺CD8⁻ cells, express the CD4 molecule, and after activation in the presence of dexamethasone, 60% of them, i.e., 43/(43 + 29), also express the CD8 antigen. The RT7ᵇ⁻ population is single-positive CD8⁺CD4⁻. The following two immunofluorescence analyses confirm the previous results, but with the opposite allotypic marker. Fig. 2 E and F, show that the RT7ᵇ⁺ cells express CD8 but not CD4 antigen. It can also be observed that the RT7ᵇ⁻ is divided into CD8⁺ and CD8⁻ cells. Again, some of the initial CD4 single-positive cells express the CD8 molecule, and the RT7ᵇ⁻ CD8⁺ cells remain single positive. Although 3% of the cells are CD4⁺CD8⁺RT7ᵇ⁻, these presumably represent doublets of CD4⁺ and CD8⁺ cells. However, because no quadrant of the contour plots in Fig. 2 is completely devoid of cells, there is an intrinsic uncertainty in the phenotype of 2-3% of the cells examined. Consequently it is not possible to disprove that rare CD8⁺ cells can become double positive. Qualitatively, the same result was found in the absence of dexamethasone, but the size of the CD4⁺CD8⁺ population was smaller than in the presence of the hormone (data not shown).

It is evident from these results that only the CD4⁺ cells become double positive after activation, and CD8⁺ cells remain single positive. Dexamethasone does not alter this pattern, but increases the percentage of CD4⁺ cells expressing CD8, as well as the level of this expression.

Dexamethasone Induces Expression of CD8α Chains but Not CD8β Chains on Con A-stimulated CD4⁺ T Cells. To determine whether CD8β chains, as well as CD8α chains, were expressed on Con A-activated CD4⁺ T cells, use was made of mAb 341, which binds only to CD8 dimers expressing the CD8β chain (28). Rat T cells, purified from lymph nodes by rosette depletions of cells expressing surface Ig and/or class II MHC antigens, were stimulated by Con A in the presence or absence of 10⁻⁸ M dexamethasone using T cell–depleted splenocytes as a source of accessory cells. After 3 d, the cells were harvested, and two-color flow cytofluorography was carried out using anti-CD4 mAb together with either anti-CD8α (OX8) or anti-CD8 heterodimer (mAb 341). The result was unequivocal: in the presence of dexamethasone 43% of the CD4⁺ T cells reacted with the anti-CD8α mAb but none with the anti-CD8 heterodimer reagent. In contrast, 89% of the CD4⁺ cells that reacted with the anti-CD8α chain mAb also bound the mAb 341. In the absence of dexamethasone, the result was the same except that, as expected, fewer CD4⁺ T cells expressed the CD8α chain (data not shown). Incidentally, these experiments furnished further evidence that the CD4/CD8 double positives do not arise through coincidental observation of single-positive CD4⁺ and CD8⁺ T cells.

Dexamethasone Appears to Induce CD8α Chain Expression by an IL-4-independent Pathway. The data in Fig. 2 demonstrate that some CD4⁺ T cells can express CD8α chains after mitogen activation, and dexamethasone increases their frequency. A similar phenomenon has been described for cloned human CD4⁺ T cells after culture in the presence of IL-4 (38, 39). It has been reported that glucocorticoids can increase IL-4 production by murine T cells in response to antigen or anti-CD3 antibody treatment (40, 41), and our initial hypothesis was that IL-4 was responsible for the induction of CD8α expression during mitogen activation, and that the enhancing effects mediated by dexamethasone were possibly due to increased IL-4 production. To examine this possibility, rat T cells were activated with Con A alone or in combination with IL-4 or dexamethasone, or with both reagents together. Fig. 3 shows that after Con A activation, the frequency of double-positive cells obtained after dexamethasone treatment (Fig. 3 B) was greater than that obtained with IL-4 (Fig. 3 C). Furthermore, IL-4 suppressed the generation of CD4⁺CD8α⁺ cells by dexamethasone, as observed after dexamethasone + IL-4 treatment (Fig. 3 D), and also reduced that obtained after Con A treatment alone (Fig. 3 A and C). Although a wide range of IL-4 concentrations were tested, it was not possible to demonstrate a positive effect of IL-4 on the induction of double-positive cells. Control supernatants, from mock-transfected CHO cells, were without effect, as were rat rIL-2 and rIFN-γ (data not shown).

Where IL-4 was used in these experiments it was added at the start of the Con A stimulation so that the cytokine was in contact with the cells throughout the activation process. Two types of experiments were performed to determine whether different results might be obtained if the T cells were activated before the addition of the cytokine. In the first of these, lymph node cells were incubated with Con A for 3 d, washed, and incubated for another 3 d in rat rIL-2. In different wells the IL-2 was supplemented with either 10⁻⁸ M dexamethasone, rat rIL-4, or a mixture of these two. The percentage of CD4⁺CD8α⁺ cells in the various cultures was as follows: in IL-2 alone, 16%; in IL-2 + dexamethasone, 31%; in IL-2 + IL-4, 5%; and in IL-2 + IL-4 + dexamethasone, 14%. In the second type of experiment lymph node T cells were stimulated with Con A for 3 d alone gave 16% of double positives, and this was reduced to 6% by the addition of IL-4 at the initiation of the culture. When the addition of IL-4 was delayed by 24 h the frequency of double positives rose to 11%. The addition of 10⁻⁸ M dexamethasone to the Con A cultures increased the frequency of double-positive CD4⁺ T cells to 29%, but the inclusion of IL-4 at the initiation of the culture reduced this figure to 11%. Again, delaying the addition of the IL-4 by 24 h reduced the level
of inhibition: 20% of cells were CD4+CD8α+ in these conditions.

Consequently, the data do not support the possibility that IL-4 was responsible for the CD8α induction during Con A activation in these experiments or for the enhancing effects of dexamethasone, but, contrary to our expectation, the lymphokine acted as a negative modulator of CD8α chain induction. The effect of delaying the addition of the IL-4 was to reduce its inhibitory effect but not to reverse it.

Isolated CD4+ Cells Can Be Induced to Express the CD8α Antigen. The preceding data show that CD8α chains can be detected on the surface of CD4+ cells after mitogen activation. However, because all the previous results were obtained in the presence of CD8+ cells, this finding could in principle be explained by the passive uptake, by the CD4+ cells, of CD8 antigen released by the CD8+ T cell subpopulation. The generation of a soluble form of the CD8 molecule during T cell activation has been described (42), so that this trivial explanation for the results did not seem untenable. To examine this possibility, CD4+ T cells were isolated, activated with mitogen using T cell-depleted splenocytes as feeder cells, and their phenotypes analyzed after a 3-d incubation period. Immunofluorescence analysis revealed that, under these conditions, activated CD4+ cells expressed CD8α on their membrane (Fig. 4 A), and at a similar level to that observed when CD8+ cells were present at the start of the culture (Fig. 3 A). Fig. 4 B shows the increase in the expression of CD8α chain promoted by dexamethasone. As in the previous experiments, IL-4 was a negative modulator of expression both in the absence (Fig. 4 C), and in the presence of the glucocorticoid (Fig. 4 D). Since, in this experiment, the CD8+ population was removed from the accessory cells and CD4+ T cells, the possibility that CD8 antigen is acquired passively by CD4+ cells can be discarded. Although in principle the existence of some very small contamination by CD8+ cells cannot be excluded, these cells would need an extremely high rate of soluble CD8 production to account for the high level of CD8 expression in highly purified CD4+ cells, comparable with the level of expression when unfractionated cells are analyzed.

These data are in contrast to a previous report with human T cells, where CD8 expression on CD4+ cells required the presence of irradiated CD8+ cells, or some conditioned supernatant from these cells. In these experiments it was not suggested that the CD8 antigen was passively acquired, and the demonstration of the biosynthesis of CD8 molecules by CD4+ T cells supported this conclusion (10).

Expression of the CD8 Gene in Activated CD4+ T Cells.

Figure 3. Effect of rat rIL-4 on the dexamethasone-induced expression of CD8α chains on CD4+ T cells. Rat T cells were stimulated with Con A for 72 h in the absence (A and C) or presence (B and D) of 10−8 M dexamethasone. The effect of including rat rIL-4 in the culture medium is shown in C and D. After culture the cells were prepared for two-color flow cytometric analysis using anti-CD4 and anti-CD8 mAbs. The histograms represent the fluorescence profiles of the CD4+ cells. The numbers on these histograms are the percentages of the CD4+ cells that also express CD8α chains.

Figure 4. CD8+ T cells are not required for the induction of CD8α chain expression on activated CD4+ T cells. Purified CD4+ T cells were activated by Con A for 72 h in the presence of T cell-depleted accessory cells. For the data shown in B and D, dexamethasone was included in the culture medium, and in some cases (C and D) rat rIL-4 was also added. As in Fig. 3, the numbers on the histograms are the percentages of the CD4+ cells that also express CD8α chains.
To provide further evidence for de novo synthesis of CD8α by CD4+ cells during activation, and to characterize at what level dexamethasone and IL-4 exert their effects, CD8α chain mRNA expression was analyzed by Northern blot in a cell culture system similar to that used in Fig. 4. As shown in Fig. 5, CD8α chain mRNA was detected from cultures of CD4+ T cells stimulated with Con A and the level of expression was markedly increased by the inclusion of dexamethasone. Furthermore, in accordance with the cell surface phenotyping data, the presence of IL-4 greatly diminished CD8α chain mRNA synthesis. It appears that both dexamethasone and IL-4 influence the transcription of the CD8α chain gene or change the rate of degradation of the mRNA.

Differential Expression of CD8α Chains on Activated OX22lowCD4+ and OX22highCD4+ Cells. CD4+ T cells in the rat can be divided into two phenotypical and functional subpopulations by the mAb OX22, which recognizes a restricted epitope on the CD45 molecule. The OX22lowCD4+ subset mediates graft-vs.-host reactions but provides little or no B cell help for secondary antibody responses. After activation it produces IL-2 and IFN-γ, but expresses little IL-4 mRNA. In contrast, reciprocal activities and lymphokine production are found for the OX22highCD4+ subset, which provides help in secondary B cell responses, produces higher levels of IL-4 mRNA, and lower levels of IL-2 and IFN-γ (23, 31, 43).

Experiments were carried out to determine whether both subsets of CD4+ cells express CD8α chains during activation and whether dexamethasone regulates this induction. As Fig. 6, C and E, show, OX22lowCD4+ cells expressed higher levels of CD8α than did OX22highCD4+ cells after mitogen activation. However, dexamethasone increased CD8α expression by both subsets, with the OX22low cells again expressing higher levels of CD8α than the OX22high ones (Fig. 6, D and F). Fig. 6, A and B, show the level of CD8α expression, after Con A and Con A plus dexamethasone treatment, on an unfractonated CD4+ culture, labeled with OX22 and OX32 mAbs as for the sorted fractions, but not separated on the FACS II. These unfractonated CD4+ cells expressed on their membranes slightly higher levels of CD8α chain than the corresponding isolated OX22highCD4+ cells (Fig. 6, C and D).

Expression of CD8α on the Rat CD4+ MBP-3 Clone. The results presented in the previous sections of this paper were obtained after polyclonal activation of fresh lymphocytes. To examine the phenomenon of CD8α chain induction during antigen-dependent activation, use was made of the CD4+ MBP-specific clone, MBP-3 (34). Fig. 7 shows the results after 3 d of stimulation with specific antigen: CD8α was expressed at low levels after activation (Fig. 7 A) and this weak expression decreased progressively during the resting stage in IL-2-containing medium until it reached an undetectable level (data not shown). Activation by antigen in IL-4-containing medium produced a further slight increase in CD8α expression (compare Fig. 7, A and B), but this was less than that observed with dexamethasone, either at the 10−4 or 10−6 M concentration (Fig. 7, C and D). However, with the clone, IL-4 did not antagonize the effect of dexamethasone as it did with freshly stimulated, polyclonally activated cells (data not shown). After several rounds of stimulation in the presence of Con A and Con A + dex, the expression of CD8α chains was further increased (Fig. 7 B).
of IL-4 or 10^{-8} M dexamethasone (both cultures supplemented with IL-2), the levels of CD8α expression were similar to those shown in Fig. 7, B and C, respectively, indicating that prolonging the time in culture did not lead to a further increase in CD8α expression in the presence of IL-4 or dexamethasone.

**Discussion**

In this study, data are presented that show a glucocorticoid-mediated enhancement of CD8α chain expression by CD4⁺ T cells during activation. Experiments were designed to exclude the possibility that the observed coexpression of CD4 and CD8α molecules during mature T cell activation was artificial. The results of these experiments indicated that alternative explanations, such as doublet formation during mitogen activation or passive uptake of soluble forms of the CD8 molecule by CD4⁺ cells, were not responsible. The remaining trivial explanation, that the increased number of cells coexpressing CD4 and CD8α in dexamethasone cultures was due to selective expansion or preferential survival of a preexisting CD4⁺CD8α⁺ population, can also be discarded: the proliferation and cell recoveries in Con A and Con A + dexamethasone cultures were quite similar. For example, in one experiment, stimulation of the cells by Con A alone lead to a doubling of cell numbers after 3 d, while the inclusion of dexamethasone at concentrations of 10^{-8} and 10^{-6} M yielded, respectively, an increase to 280% and to 160% of the initial cell numbers. Furthermore, the frequency of double-positive cells was not significantly changed by removing cells expressing CD8 antigen before culture with mitogen. Such a depletion would be expected to also remove any preexisting CD4⁺CD8α⁺ cells. Finally, CD8α expression by an antigen-activated CD4⁺ T cell clone was also enhanced by dexamethasone. The assumption that the anti-CD8α chain mAb was not detecting some unexpected crossreactivity was substantiated by the finding that mRNA for CD8α was detectable at elevated levels in cultures containing double-positive cells. It may be noted also that human T cell clones of the phenotype CD4⁺CD8α⁺ have been described (44).

It is important to mention some apparent discrepancies with respect to other reports. IL-4 has been shown to induce the expression of CD8 molecules on human CD4⁺ clones after alloantigen (38) and PHA (39) activation. These studies are not closely comparable with the majority of the ones described here because they examined the effects of long-term exposure of clones to IL-4, whereas we analyzed the phenotype of freshly isolated cells after a 3-d activation period. However, the single rat CD4⁺ T cell clone that we studied did express elevated levels of CD8α after several rounds of activation in the presence of IL-4, and this result is not qualitatively at variance with the published data on the human clones. There is, however, a quantitative difference in that in the published data the human clones expressed much higher levels of CD8. At present it is unclear whether there are real differences in the response of human and rat CD4⁺ T cells to IL-4 or whether methodological differences can account for the apparent discrepancies. Further studies are necessary in both species.

Another discrepancy is the derivation of CD4⁺CD8α⁺ cells from purified rat CD4⁺ T cells, without adding any exogenous factor. Blue et al. (10) reported the inability of purified human CD4⁺ cells to coexpress the CD8 molecule without the addition of irradiated CD8⁺ cells or some conditioned supernatant from CD8⁺ cells. The nature of this conditioned supernatant has not been described, and it is possible that the T cell-depleted splenocytes that we used as accessory cells also provide it. However, there is no consensus on the need for the presence of CD8⁺ T cell products in the induction of double-positive T cells, and the situation remains unresolved.

The functional activities of the dexamethasone-induced double-positive T cells described in this paper have yet to be determined, but it is notable that some of the reported suppressor clones in human (45) and mouse (46, 47) are CD4⁺CD8⁺ or become so on activation (48). In view of this correlation between phenotype and function, it is tempting to associate the phenotypic changes induced in CD4⁺ cells by dexamethasone, with the immunosuppressive properties of glucocorticoids. One CD4⁺CD8⁺ suppressor clone (48) was generated in the presence of cyclosporine A, another immunosuppressive agent. CsA has been shown to induce in

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**Figure 7.** Expression of CD8 on a rat T cell clone and the effects of dexamethasone and IL-4. A rat CD4⁺ T cell clone, specific for guinea pig MBP, was stimulated for 72 h with specific antigen. The culture medium was supplemented with IL-4 or dexamethasone as follows: (A) stimulation with antigen alone; (B) antigen plus rat rIL-4; (C) antigen plus 10^{-8} M dexamethasone; (D) antigen plus 10^{-6} M dexamethasone. The interrupted traces and the continuous line traces are the fluorescence histograms obtained using, respectively, a nonreactive mAb (MRC-0X21) and an anti-CD8 mAb (MRC-0X8) to label the cells. The percentages given indicate the frequency of cells specifically labeled by the anti-CD8 mAb, and the numbers in parentheses are the channel numbers for the peaks in the fluorescence histograms.
vivo a double-positive population in the blood of heart allografted rats that were tolerant of their allografts (13). Hence, it seems that both immunosuppressive drugs, CsA and glucocorticoids, are able to induce a CD4⁺CD8⁺ population, and this phenotype is also expressed in many suppressor clones. If coexpression of CD4 and CD8 is a marker of suppression, and this phenotype is also expressed in many suppressor clones. If coexpression of CD4 and CD8 is a marker of suppression, then there may be major significance in the recent report that the frequency of MBP-specific T cell lines that were CD4⁺CD8⁺ was much higher in normal individuals than in patients with multiple sclerosis (49). In this context it is notable that when rat CD4⁺ T cells were fractionated on the basis of their level of expression of the OX22 epitope it was the population that was OX22low that produced the greater frequency of CD4⁺CD8α⁺ double positives on activation (Fig. 6). Cells with the OX22lowCD4⁺ phenotype have been shown to prevent autoimmunity in two model systems in the rat (50, 51), but it is not known at present whether it is those cells that become double positive on activation that mediate this protective function. Further, because there is probably a higher frequency of memory T cells in the OX22low subset (51), it may be that only memory CD4⁺ T cells can be induced to express CD8α⁻ chains. In any event, a study of the physiological significance of the ability of glucocorticoids to enhance CD8α⁻ chain expression on CD4⁺ cells is now required.

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