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CD49d Overexpression and T Cell Autoimmunity

Ru-Ran Mo,* Julie K. Eisenbraun,* Joanne Sonstein,† Ronald A. Craig,‡ Jeffrey L. Curtis,† Lloyd M. Stoolman,‡ Jun Chen,* and Raymond L. Yung2*§

D10.G4.1 (D10) cells, a murine conalbumin-reactive Th2 cell line, made to overexpress the β2 integrin LFA-1 by pharmacological manipulation or by transfection become autoreactive and are capable of inducing in vivo autoimmunity. However, whether this is specific to LFA-1 and whether overexpression of other T cell integrin molecules has the same effect are unknown. We examined the functional consequences of T cell CD49d (α4 integrin) overexpression by transfecting murine CD49d cDNA into D10 cells. Similar to the LFA-1-transfected cells, the CD49d-overexpressing T cells are autoreactive and proliferate in response to APCs in an MHC class II-dependent manner in the absence of nominal Ag. Additionally, CD49d overexpression is associated with increased in vitro adhesion to endothelial cells and increased in vivo splenic homing. However, in contrast to LFA-1 overexpression, increased T cell CD49d expression is not associated with autoreactive cytotoxicity or the ability to induce in vivo autoimmunity. In addition to the novel observation that CD49d overexpression is sufficient to induce T cell autoreactivity, our results also support the hypothesis that the ability to induce in vivo autoimmunity is related to T cell cytotoxicity and not to T cell proliferation function in the D10 murine adoptive transfer model of autoimmunity. The Journal of Immunology, 2003, 171: 745–753.

The drug-treated cells lose the requirement for nominal Ags and will proliferate in response to MHC class II molecules on APCs alone. Importantly, the LFA-overexpressing cells will induce apoptosis in syngeneic macrophages (MD) without specific Ag (6, 8–10), thereby providing a potential source of autoantigens that may then take part in the autoimmune process. Adoptive transfer of the drug-treated autoreactive T cells into syngeneic murine hosts results in the development of in vivo autoimmunity characterized by the presence of autoantibodies, immune complex glomerulonephritis, and autoimmune liver and lung diseases (6, 8–10). This murine system appears to be independent of T cell cytokine profile, because both drug-treated Th1 and Th2 cells are autoreactive and can induce autoimmunity (11). Interestingly, the spleen appears to be critical in this model, because splenectomized mice receiving the autoreactive cells do not develop evidence of autoimmune disease (12). To directly show that the observed in vitro T cell autoreactivity is due to increased T cell LFA-1 expression, we transfected the human CD11a cDNA into a tetanus toxoid-reactive human CD4+ T cell line (7) and the murine CD18 cDNA into D10 cells (9). In both human and murine T cells, the resulting LFA-1 overexpression is associated with the induction of in vitro autoreactivity, similar to the drug-treated T cells. Finally, we showed that adoptive transfer of the CD18-transfected D10 cells resulted in the development of autoantibodies (anti-ssDNA, anti-dsDNA) and immune complex glomerulonephritis in syngeneic AKR mice. However, the underlying mechanisms by which the autoreactive D10 cells induce in vivo autoimmunity are unclear. It is also unknown whether the ability to induce T cell autoreactivity and in vivo autoimmunity is unique to LFA-1 and whether overexpression of other T cell integrin, such as the α4 integrin, will have similar effects. This is an important question because the α4 integrins have been implicated in a number of human (13–15) and animal (16–18) models of autoimmune diseases. In this report, we examined the role of α4 integrin overexpression in autoimmunity by stably transfecting a cloned murine T cell line with a CD49d cDNA construct. We then asked whether the transfected cells become autoreactive and whether adoptive transfer of the α4-overexpressing T cell will induce in vivo autoimmunity.

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3 Abbreviations used in this paper: VLA-4, very late activation Ag-4; McAb, monoclonal antibody; EMFDA, 5-chloromethylfluorescein diacetate.

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Materials and Methods

**Mice and peritoneal Mφ isolation**

Six- to 8-wk-old female AKR (Iak) mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and maintained in a specific pathogen-free environment. Peritoneal Mφ were obtained by i.p. thioglycolate (BD Biosciences, San Diego, CA) injection and harvested 3 days later as previously described (6, 8, 9).

**T cell and endothelial cell culture**

D10 cells (19) obtained from the American Type Culture Collection (Manassas, VA) were maintained by challenging them weekly with irradiated syngeneic AKR/J mouse splenocytes and conalbumin (8, 9). Because of a report that the D10 cell line may contain an autoreactive subset (20), the D10 cells were subcloned by limiting dilution at ≤0.2 cells/well, and a nonautoreactive subclone was selected. All cells used for functional or cytotoxic analysis were studied 6 days after challenge. Dermal microvascular endothelial cells from AKR mice were isolated according to a previously published protocol (21). Identity of the isolated endothelial cells was confirmed by their typical cobblestone appearance and Von Willebrand factor (21) and CD34 (22) expression.

**Generation of the pSub2-CD49d constructs and transfection into D10 cells**

The expression vector pSub2 that we used previously to generate the D10/CD18 transfectants (9) was donated by Dr. M. Clarke (University of Michigan). The pSub2 polylinker site was cleaved with EcoRV, and EcoRI (NotI) adapters (Life Technologies, Gaithersburg, MD) ligated to the blunt ends. A full-length 4-kb cDNA-encoding murine CD49d in PBSK plasmid kindly donated by Dr. I. Weissman (Stanford University, Stanford, CA) (23), was excised with NotI and cloned into the NotI sites generated by the adapters. The cDNA orientation in the subclones was determined by digestion with BgIII, and a plasmid containing the complete cDNA in the sense and antisense (control) direction was selected. The construct was linearized by digestion with Sca1, and D10 cells were transfected by electroporation as before (7, 9). Stable transfecteds were selected and maintained by culturing in medium containing 250 μg/ml Geneticin (G418) (Life Technologies). Finally, the transfecteds were subcloned by limiting dilution at ≤0.2 cells/well.

**DNA isolation and Southern analysis**

Southern analysis was performed using previously published protocols (7, 9). Briefly, 20 μg of genomic DNA from control or transfected D10 cells were digested with NotI (Boehringer Mannheim, Mannheim, Germany), fractionated through agarose gels, and transferred to nylon filters (Magnagraph; Micro Separations, Westborough, MA). The filters were probed using a 32P-labeled full-length CD49d cDNA, excised from pSub2 with NotI, and exposed to x-ray film.

**Flow-cytometric analysis**

Cultured T cells were stained with mAbs conjugated with FITC or PE including anti-CD3, -TCR, -CD18, -CD28, -CD29, -β2, -CD49d, -CD62L, -CD49d/β2 (DATK32) and goat anti-mouse Ig (Coulter, Hialeah, FL) as previously described (6–9), and analyzed on a Coulter ELITE flow cytometer. All of the mAbs used in these studies were purchased from BD PharMingen (San Diego, CA) unless stated otherwise.

**Proliferation assay**

Proliferation assays were performed as described (6–9), using irradiated AKR splenocytes as APCs. For the inhibition studies, the CD49d-transfected D10 cells were preincubated with mAbs against CD49d, CD29, β2, CD18, Iak, Iab/d, or control Ig Abs for 30 min at 37°C, before coculturing with the APCs.

**Cytotoxicity assay**

For cytotoxic response, peritoneal Mφ were labeled with 51Cr by culturing 10 × 10^6 cells with 100 μCi of 51Cr-labeled Na2Cr2O7 in 1 ml at 37°C for 1 h. The 51Cr-labeled D10 cells were then allowed to adhere to the endothelial cells for 30 min at 37°C. The adhered cells were then lysed with Nonidet P-40, and radioactivity was counted by a scintillation counter (6, 9). For the functional blockade experiments, the CD49d-transfected D10 cells were preincubated with anti-CD49d, -CD29, or β2 mAbs for 30 min at 37°C and washed three times with RPMI 1640, before adhesion to the endothelial cells.

**Flow adhesion assay**

The parallel-plate flow chamber used in the present study has been described previously in detail (24, 25). Microvascular endothelial monolayers on coverslips were stimulated with TNF-α (2.5 ng/ml) for 16 h at 37°C and mounted on an inverted microscope. A circular glass window in the top plate allows direct examination of the monolayer during the experiment. The monolayer was perfused for 5–10 min with perfusion medium. A total of 10^5 cells per milliliter of perfusion medium of the CD49d-transfected and untransfected D10 cells were drawn through the chamber at 2.0 dynes/cm² for 5 min and then decreased to 0.2 dynes/cm² for 5 min. Leukocyte adhesion was determined by counting the number of T cells in 20 randomly selected high-power microscope fields during the final minute at each level of flow.

**Western blot**

Tyrosine phosphorylation of anti-CD3-stimulated D10 and D10 transfec- tants was determined as before (26). Briefly, the cells were stimulated with anti-CD3 (10 μg/ml) for 10 min and cross-linked with goat anti-rat IgG. Proteins from the cells were resolved on 10% SDS-polyacrylamide gel and transferred to nitrocellulose-1 membrane. The membrane was then incubated with HRP-conjugated anti-phosphotyrosine Ab (Chemicon International, Temecula, CA) and anti-mouse IgG (Chemicon International). Detection was performed using the ECL system (Amersham, Arlington Heights, IL) as before (27). The membrane was then stripped and probed with anti-β2-actin Abs (Chemicon International).

**T cell adoptive transfer model**

To induce autoimmunity, 5 × 10^6 CD49d-transfected and untransfected D10 cells were injected i.v. via the tail vein into 11 and 10 6-wk-old AKR mice, respectively, every 2 wk for a total of six injections. This is the same protocol we have used successfully to induce autoimmunity using LFA-1-overexpressing D10 cells (6, 8–11) and is based on the murine graft-vs-host T cell adoptive transfer model (28). The mice were sacrificed 4 wk after the last adoptive transfer. A complete necropsy was done.

**ELISA**

Splenic B cells from AKR/J mice were isolated by MACS (Miltenyi Biotech, Auburn, CA) using CD19 microbeads (27) and cocultured with the D10 transfectants for 72 h as before (29). Anti-ssDNA Abs and the supernatant were measured by ELISA using previously published protocols (8–11).

**In vivo splenome hoging**

Untransfected and CD49d-transfected D10 cells were labeled with CellTracker Green 5-chloromethylfluorescein diacetate (CMFDA, CFSE, both from Molecular Probes, Eugene, OR), or PKH26 green fluorescent polymer (Sigma-Aldrich, St. Louis, MO) using the protocols provided by the manufacturers (12). Viability of the cells was determined by trypan blue exclusion and was similar in both the labeled and unlabeled cells. The labeled transfected and untransfected cells were injected i.v. via the tail vein, and the mice were killed 24 h later. Labeled D10 cells were detected by staining splenocytes with FITC- or PE-conjugated anti-CD4 and then enumerating cells expressing both CD4 and CMFDA or CFSE as before (12).

**Statistical analysis**

The difference between means was tested using Student’s two-tailed t test.

**Results**

**Characterization of CD49d-transfected D10 cells**

D10, which has previously been shown to induce autoimmunity after transfection with the CD18 cDNA (9), was first subcloned by limiting dilution, and an Ag-reactive subclone was selected. A full-length cDNA-encoding murine CD49d was cloned into the mammalian expression vector pSub2, linearized by digestion with Sca1, MA). Untransfected and CD49d-transfected D10 cells were labeled with 51Cr by culturing 10 × 10^6 cells with 100 μCi of 51Cr-labeled Na2Cr2O7 in 1 ml at 37°C for 1 h. The 51Cr-labeled D10 cells were then allowed to adhere to the endothelial cells for 30 min at 37°C. The adhered cells were then lysed with Nonidet P-40, and radioactivity was counted by a scintillation counter (6, 9). For the functional blockade experiments, the CD49d-transfected D10 cells were preincubated with anti-CD49d, -CD29, or β2 mAbs for 30 min at 37°C and washed three times with RPMI 1640, before adhesion to the endothelial cells.
and transfected into the D10 subclone by electroporation, and stable transfectants were selected using G418. The transfected cells were subcloned by limiting dilution (0.2 cell/well). Incorporation of the full-length CD49d cDNA was confirmed by Southern analysis (Fig. 1A). Experiments were then performed to determine the effect of CD49d transfection on the cell surface expression of adhesion and costimulatory molecules by flow-cytometric analyses. The cultured T cells were stained with anti-CD3, -TCR, -CD18, -CD28, -CD29, -CD49d, -β7, and -CD62L (Fig. 1B). The results show that D10 cells normally express a low level of CD49d, CD29, and β7 that is only slightly above background staining. Transfection of CD49d cDNA caused a 10-fold increase in CD49d expression in D10 cells but no significant change in the expression of other adhesion or costimulatory Ags. Interestingly, the CD49d transfectants showed increased expression of the corresponding β7 chain but not the CD29 chain, suggesting that the overexpressed α4 chains are preferentially forming a heterodimer with the endogenous β7 chains. To confirm this, the transfected cells were stained with the DATK32 mAb that is specific for the α4β7 heterodimer (Fig. 1C). The reason why the CD49d-transfected D10 cells overexpress the corresponding β7 chain but not the CD29 chain is unclear. However, examination of 15 other CD49d-transfected clones all showed similar increased expression of β7 and not the CD29 chain. This suggests that the selective increase in the β7 chain in response to increased CD49d expression is not a random but a regulated process in D10 cells.

**T cell proliferation response**

Because D10 cells overexpressing the integrin LFA-1 no longer require Ag and will proliferate in the presence of APCs alone (8–11), we determined whether CD49d overexpression has the same effect in D10 cells. Three CD49d-transfected D10 clones (clones 16, 29, and 39) were tested for autoreactive proliferation response against irradiated syngeneic splenocytes. All of the CD49d transfectants were shown to proliferate without conalbumin, similar to the CD18-transfected D10 cells (Fig. 2A). Because clone 29 most closely resembled the CD18 transfectants in its autoreactivity response, the line was chosen for further characterization and for the in vivo adaptive transfer experiment. Clone 29 was found to proliferate in the presence of APCs and suboptimal concentrations of conalbumin (Fig. 2B). In addition, the CD49d-transfected D10 cells became autoreactive and proliferated in response to autologous APCs without the addition of Ag (Fig. 2C), similar to D10 cells made to overexpress CD18 by transfection (9). The increased T cell Ag proliferation response in the transfectants is reversed by the addition of saturating amount (10 μg/ml, final concentration) of functionally blocking mAbs against CD49d, β7, or the appropriate MHC class II Ag (Fig. 2D). Anti-CD18 treatment also partially reversed the increased Ag proliferation response, consistent with the known costimulatory role that LFA-1 plays in normal T cell Ag response. Similarly, the autoreactive proliferation response of the CD49d transfectants is found to be CD49d and MHC class II dependent (Fig. 2E).

**Tyrosine phosphorylation**

We have previously shown increased signaling in LFA-1-overexpressing T cells (26). We next determined whether T cells overexpressing CD49d also have increased signaling as measured by tyrosine phosphorylation. The results show that both the CD18- and CD49d-transfected cells have increased TCR/CD3-mediated tyrosine phosphorylation of cellular protein substrates (Fig. 3).

**T cell cytotoxicity**

Earlier studies have established that untransfected D10 cells will lyse syngeneic Mφ, but only in the presence of conalbumin (8). In contrast, CD18-overexpressing D10 cells that cause in vivo autoimmunity induce apoptosis in syngeneic Mφ in vitro without Ag (8, 9) by a process that likely involves TNF-related apoptosis-inducing ligand (Apo2 ligand) and TNF-like weak inducer of apoptosis (Apo3 ligand) (30). This has led us to propose that such Mφ killing in lymphoid organs, including the spleen, may provide...
a source for autoantigens that contribute to the production of autoantibodies and in vivo autoimmunity in our D10 model. Therefore, we determined whether CD49d overexpression will also induce D10 cells to kill syngeneic Mφ in the absence of Ag. The results showed that, unlike CD18 overexpression, clone 29 CD49d transfectants do not cause increased Mφ killing compared with untransfected D10 cells (cytotoxicity index: untransfected D10 cells vs CD49d transfectants 100 vs 107 ± 9.9, mean ± SEM; p = NS; n = 5 independent experiments). Clones 16, 29, and 39 all had similar cytotoxicity response (mean ± SD: 39 ± 2, 33 ±

FIGURE 2. CD49d overexpression causes autoreactivity in D10 cells. A, Autoreactive proliferation of CD18 transfectants, three different CD49d clones (clones 16, 29, and 39), and untransfected D10 cells were compared. B, The Ag proliferation response of untransfected (D10) and CD49d transfected (clone 29) D10 cells showing that the CD49d transfectants will proliferate in the presence of suboptimal concentrations of Ag (conalbumin). C, Autoreactive proliferation response of untransfected and CD49d-transfected D10 cells in the presence of the indicated number of irradiated splenocytes. Effects of saturating amount (10 µg/ml, final concentration) of functional Ab blockade on the Ag (D) and autoreactive (E) proliferation responses of CD49d-transfected D10 cells. The results represent the mean ± SEM of quadruplicate determinations.
Adhesion to microvascular endothelial cells

Primary dermal microvascular endothelial cells were isolated from 3- to 4-wk-old female AKR mice. Vascular endothelial cells were identified according to morphological appearance, Von Willibrand factor, and CD34 expression (data not shown). In vitro adhesion function of the CD49d transfectants was assessed by adhesion assays, performed under static and flow conditions. Under static condition, four times more CD49d transfectants were found to adhere to TNF-stimulated endothelial cells compared with untransfected D10 cells (p < 0.01) (Fig. 4A). The increased adhesion of the CD49d transfectants was reversed with the addition of anti-CD49d, but not anti-β7 or CD29 Abs (Fig. 4B). Binding of CD49d transfectant under high (physiological) flow rate (2.0 dyne/cm²) was similar to that of untransfected D10 cells (data not shown). However, a 4-fold increase in binding to endothelial cells was observed under low-flow condition (0.2 dyne/cm²) (p < 0.01) (Fig. 4C). The simulated flow adhesion data confirm the results of the static adhesion assays and are consistent with the notion that α4 integrin is involved in the firm adhesion of leukocytes to vascular endothelial cells, and less in the initial contact or rolling stage of the interaction.

In vivo effects of D10 CD49d overexpression

Adoptive transfer of D10 cells made to overexpress LFA-1 by pharmacological treatment (8, 10–12) or by CD18 transfection (9) caused in vivo autoimmunity in syngeneic mice, with features that include the development of anti-ssDNA and -dsDNA Abs, immune complex glomerulonephritis, and interstitial pneumonitis. Because CD49d-overexpressing D10 cells are also autoreactive and because CD49d overexpressing T cells have been found in lupus patients with vasculitis (4), we next determined the ability of the CD49d transfectants to induce in vivo autoimmunity, using the same adoptive transfer protocol that we used previously (8–12). Interestingly, although clone 29 CD49d transfectants display similar in vitro autoreactive proliferation as the CD18 transfectants, the D10/CD49d transfectants failed to elicit an autoimmune response in syngeneic hosts. Mice receiving the CD49d-overexpressing T cells did not develop anti-ssDNA or -dsDNA Abs (Fig. 5). Total serum IgG (OD: D10 vs CD49d, mean ± SD = 0.432 ± 0.006 vs 0.436 ± 0.008; p = NS) and IgM (OD: D10 vs CD49d, mean ± SD = 0.045 ± 0.039 vs 0.015 ± 0.006; p = NS) levels were also determined by ELISA and were found to be comparable in both groups, excluding the possibility that the lack of difference in autoantibody levels is due to variability in Ig levels. Histologic analysis also failed to show evidence of autoimmune features or vasculitis in mice receiving the CD49d transfectants (Fig. 6).
ELISA

Anti-ssDNA measurements were done following in vitro cocultures of CD49d transfectants with B cells from AKR/J mice. Consistent with the in vivo data, the CD49d transfectants did not promote B cell anti-DNA response (OD, mean ± SD of quadruplicate determinations: B cell alone, 0.106 ± 0.012; B cells with D10, 0.126 ± 0.008; B cells with clone 16, 0.119 ± 0.01; B cells with clone 29, 0.117 ± 0.004; B cells with clone 39, 0.129 ± 0.08; and MRL/lpr, 3.668 ± 0.247).

In vivo splenic homing

We have previously shown that D10 homing to the spleen is critical for the development of in vivo autoimmunity in the D10 murine autoimmunity model, and splenectomy will protect the recipients receiving the LFA-1-overexpressing D10 cells from developing the disease (12). To exclude the possibility that T cell CD49d overexpression may cause decreased splenic homing by directing the CD49d transfectants to mucosal or other lymphoid organs and thus protect the recipients from developing autoimmunity, we examined the effect of CD49d expression on in vivo T cell homing. This was initially done by injecting 5 × 10⁶ CMFDA-labeled D10 cells or CD49d transfectants into AKR mice. The number of CD4⁺CMFDA⁺ cells in the spleens of the recipient mice was then determined by flow-cytometric analysis 24 h later. CD49d transfectants were found to traffic to the spleen three to four times more than the control D10 cells (p < 0.05) (Fig. 7A). It is possible that the observed increased splenic homing is the result of unintended differences in the number of cells given to the individual animal in the adoptive transfer process. To exclude this possibility, AKR mice were given an i.v. mixture of either 5 × 10⁶ CFSE (green)-labeled D10 cells and 5 × 10⁶ PKH26 (red)-labeled CD49d transfectants, or 5 × 10⁶ CFSE-labeled CD49d transfec-
tants and 5 × 10⁶ PKH26-labeled D10 cells. The number of D10 and CD49d transfectants in the spleen was then enumerated by flow cytometry 24 h later. Mice that received the CFSE-labeled D10 cells and PKH26-labeled CD49d transfectants were found to have significantly more CD49d transfectants in the spleen (Fig. 7B) compared with untransfected D10 cells (p < 0.001). Similarly mice receiving the PKH26-labeled D10 cells and CFSE-labeled CD49d transfectants were found to have significantly increased D10/CD49d splenic homing (D10 vs CD49d, mean ± SD, 87 ± 5 vs 132 ± 10 per 100,000 events; n = 8; p < 0.001).
The spleen cells were harvested 24 h later, and the percentages of CMFDA-positive cells were enumerated by flow-cytometric analysis. The results showed that significantly more CD49d transfectants homed to the spleen (p < 0.05). The results are expressed as the percentage of spleen cells that are CMFDA positive. Four AKR mice were given $5 \times 10^6$ CFSE-labeled D10 cells and $5 \times 10^6$ PKH26-labeled CD49d transfectants, and the spleens were harvested 24 h later. A significantly greater number of CD49d transfectants was found to home to the spleen (B) (p < 0.001). The results are expressed as the number of CFSE-positive or PKH26-positive events per 100,000 events during the flow-cytometric studies.

**Discussion**

In addition to their adhesion and T cell migration functions, the integrins are increasingly recognized for their role in Th cell activation (31) and in their ability to transduce bidirectional signals into and out of the cell (32, 33). T cell integrins have a major role in the initial Ag-independent adhesion to APCs. The initiation of the subsequent Ag-specific T cell proliferative response involves the engagement of the TCR/CD3 complex with antigenic peptide presented by a MHC molecule on the APC. However, this interaction is generally not sufficient to stimulate resting T cells. Additional costimulatory signals, such as those provided by CD7/CD28, LFA-1/ICAM-1 (34–36), or VLA-4/VCAM-1 (34) interactions, are necessary for optimal T cell effector functions. Adhesion through VLA-4 triggers tyrosine phosphorylation of a 105-kDa protein in lymphocytes (37) and may both promote (38) and inhibit (39) cell death. It appears that integrin- and CD28-mediated stimulations are distinct but may complement each other at different stages of the Th cell activation cascade (31). Compared with LFA-1 and VLA-4, the role of lymphocyte Peyer’s patch adhesion molecule-1 in Th cell activation and the resulting downstream functional consequence are less clear. The subpopulation of activated or memory T cells has been shown to express a high level of $\alpha_4\beta_2$ (40). Although in vitro stimulation of resting T cells is associated with a late up-regulation of integrin cell surface expression (41), early down-regulation of $\alpha_4\beta_2$ is also seen in activated T lymphocytes upon recognition of alloantigen or in the presence of superantigen (42).

In the current study, we determined the effects of CD49d overexpression on T cell autoimmunity by stably transfecting the $\alpha_4$ cDNA into D10 cells. In addition to CD49d overexpression, we found that transfecting the $\alpha_4$ cDNA into D10 cells results in increased cell surface expression of the corresponding $\beta_2$ but not the CD29 chain. The reason for the selective $\beta_2$ up-regulation in D10 cell is unknown but may be related to the observation that increased $\beta_2$ expression is a phenotype of memory T cells. Others have reported that transfecting the human CD49d cDNA into mouse L cells could induce de novo surface expression of host $\beta_2$ and $\beta_7$ chains, suggesting that the $\alpha_4$ subunit requires the $\beta$ subunit for surface expression (43). Our current results show that D10 cells overexpressing the $\alpha_4$ integrin also lose the requirement for nominal Ag and will proliferate in response to APCs alone, similar to the LFA-1-overexpressing T cells. The possibility that CD49d transfection caused overexpression of other adhesion or costimulatory molecules was excluded by flow-cytometric cell staining. The autoreactivity is MHC class II specific, as anti-Ia/b will reverse the D10 autoreactivity. Interestingly, the autoreactivity is also abolished by treatment with either anti-CD49d or anti-$\beta_2$ Abs, suggesting that both the heterodimer subunits are involved in this reaction.

We did not determine the basis by which overexpression of CD49d induced T cell autoreactivity in this report. Potential mechanisms include increased costimulatory signals and/or overstimulation of normally low-affinity TCR-IA interactions. Recently it was shown that CD49d itself can also act as a cellular ligand for both $\alpha_4\beta_2$ and $\alpha_4\beta_7$, with functional consequences (44). Increased CD49d expression may therefore also provide an increased costimulatory signal to the adjacent CD49d transfectant cells. We have previously examined the mechanism in T cell autoreactivity caused by overexpression of CD18 and showed that CD18 overexpression permits TCR signal transmission in response to a normally subthreshold stimulus presented by Mφ, consistent with overstimulation of TCR-IA interaction. In addition, CD18 overexpression also causes increased tyrosine phosphorylation, although this alone is not sufficient to induce a proliferative response to a low level of stimuli (26). Therefore, it is reasonable to postulate that overstimulation of TCR-IA also occurs in the CD49d overexpressing D10 cells.

In contrast to the LFA-1-overexpressing D10 cells, the $\alpha_4$ transfectants do not kill syngeneic Mφ and are incapable of inducing autoimmunity in vivo. Given the important role of the spleen in our T cell adoptive transfer model system, one potential explanation would be that the $\alpha_4$ overexpression may result in decreased trafficking of the autoreactive cells to the spleen, possibly through increased homing to other lymphoid organs, such as those in the gut mucosa. However, our data showed that $\alpha_4$ overexpression is associated with significantly increased T cell splenic homing. The idea that CD49d may participate in tissue- or organ-specific T cell trafficking is supported by existing literature. Naive-phenotype T cells recirculate through both peripheral and mucosal sites, whereas tissue-specific recirculation is a property of memory-phe- notype (often $\alpha_4\beta_2^{high}$) cells (45). Others have also shown that $\alpha_4\beta_2^{high}$ memory T lymphocytes home efficiently to the spleen (46). Mucosal addressin cell adhesion molecule-1 is expressed by the sinus-lining cells closest to the splenic lymphoid white pulp (47). The entrance and retention of T and B cells into the white pulp is a highly organized process (48, 49). The location of mucosal addressin cell adhesion molecule-1 in the spleen suggests that interaction with $\alpha_4\beta_2$ may be an important determinant in lymphocyte splenic homing. In addition, high $\alpha_4\beta_2$-expressing CD4$^+$ T cells have also been described in the spleen (50).

The reason for the differential ability of the LFA-1- and CD49d-overexpressing D10 cells to induce in vivo autoimmunity is uncertain. The ability of the autoreactive LFA-1-overexpressing, but not the CD49d-overexpressing, D10 cells to cause Mφ apoptosis may help explain the difference. D10 cells have been shown to be capable of inducing apoptosis in syngeneic Mφ through Fas-independent pathways (30). Although how LFA-1 overexpression increases D10 cytotoxicity is unclear, many investigators have reported an important role of LFA-1 in modulating T cell cytotoxic functions (51–54). In contrast, the $\alpha_4$ integrins have not been implicated in this process. Increased monocyte apoptosis has been reported in lupus patients, and it has been proposed that, if this occurs in the appropriate lymphoid organ such as the spleen, it may provide a source of antigenic nucleic acids that contributes to the autoimmune process in these patients (30, 55). Future experiments...
comparing the ability of autoreactive T cells to induce in vivo autoimmune in control and apoptosis knockout mice may provide the definitive answer to this question. Another approach is to generate specific integrin transgenic mice. However, this approach may not be feasible, because integrin has been shown to be involved in the thymic deletion of potentially self-reactive T cells (56). Therefore, it is likely that the integrin-overexpressing autoreactive T cells will be deleted in the transgenic animals.

In summary, we have shown that the ability to induce T cell autoreactivity is not limited to LFA-1, but that overexpression of other integrins such as CD49d is also sufficient to allow T cells to lose the requirement for Ag to proliferate, presumably through overstabilization of TCR-ligand interaction. However, in contrast to LFA-1, increased T cell CD49d expression is not sufficient to induce killing of APCs or in vivo autoimmunity. This will suggest that the ability of autoreactive T cells to cause autoimmune disease is dependent on their ability to induce apoptosis of APCs in lymphoid organs such as the spleen.

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References


