Immunology 1994 **82** 301–305

Anti-CD11a prevents deletion of self-reactive T cells in neonatal C57BR mice

J. QUDDUS,* A. KAPLAN* & B. C. RICHARDSON*† *Department of Medicine, University of Michigan, Ann Arbor and †Ann Arbor Veterans Administration Hospital, Ann Arbor, Michigan, U.S.A.

SUMMARY

The process of thymic maturation permits development of T cells expressing receptors which recognize self-major histocompatibility complex (MHC) determinants, but deletes T cells recognizing self-MHC determinants with high affinity. This selection process is evolutionarily conserved, and presumably serves in part to prevent the release of autoreactive cells. However, the mechanisms involved in the selection process, and the molecules required are incompletely characterized. Lymphocyte function-associated antigen-1 (LFA-1) is an accessory molecule important in T-cell activation, is involved in thymocyte–epithelial cell binding, and contributes to the maturation of $CD4^-$ $CD8^-$ thymocytes to the $CD4^+$ $CD8^+$ stage. In this report we have investigated whether LFA-1 also contributes to the thymic deletion of potentially self-reactive cells. Neonatal C57Br mice were injected with amounts of a monoclonal antibody to LFA-1 that saturated thymic binding sites, then splenocytes were examined for T cells expressing receptors normally deleted in the thymus. The results demonstrate that $V\beta17a^+$ T cells, normally deleted in this strain, can be detected in the spleen following administration of anti-LFA-1, thus supporting the hypothesis that LFA-1 also contributes to negative selection. The potential significance of LFA-1 involvement in multiple thymic maturation events is discussed.

INTRODUCTION

Current models propose that the binding affinity between T-cell receptors (TcR) and major histocompatibility complex (MHC)–peptide complexes is a crucial factor in determining whether or not a T cell responds to the peptide presented in the binding cleft of MHC molecules.[1,2] This concept is supported by extensive experimental evidence (reviewed in ref. 1), and is consistent with fundamental principles of receptor–ligand interactions. However, the interaction between the TcR, MHC molecules and antigenic peptides is often not sufficient for activation, and activation may require additional stabilization, or possibly co-stimulatory signals, provided by accessory molecules binding their respective ligands. These accessory receptor–ligand interactions include CD4 and Ia,[3] CD2 and CD58[4,5] and lymphocyte function-associated antigen-1 (LFA-1) (CD11a/CD18) and intracellular adhesion molecule-1 (ICAM-1) (CD54).[4,6]

Recent evidence suggests that LFA-1–ICAM-1 interactions are particularly important when the number of class II MHC molecules is limiting,[7] or when the interaction between the TcR and class II molecules is of low affinity.[8,9] For example, our laboratory has reported that T-cell activation by self-MHC determinants alone, presumably a relatively low affinity TcR–MHC interaction, is more strongly dependent on LFA-1–ICAM-1 interactions than is activation by the higher affinity interaction of the TcR with self-MHC plus antigen.[9] Others have proposed a similar role for CD4 in low-affinity interactions.[10] Together, these reports suggest that T-cell activation is not only dependent on the affinity of binding between the TcR and the MHC–peptide complex, but also on the stabilizing or signalling contributions of accessory molecules such as CD4 and LFA-1.

The concept that T-cell activation reflects both TcR–MHC binding affinity and 'help' from accessory molecules may have relevance to thymic maturation. Cortical thymocytes undergo a two-step selection process in which immature thymocytes with receptors recognizing 'self'-MHC determinants are first positively selected, then negatively selected (deleted) if they recognize 'self' strongly enough to be autoreactive. Both steps involve TcR recognition of self-MHC determinants, as well as contributions from accessory molecules. Evidence for the participation of MHC molecules comes in part from experiments in which allele-specific anti-Ia and monoclonal antibody (mAb) administered to neonatal mice prevent the positive selection of T cells recognizing the targeted Ia molecules.[11–13] Similar experiments have shown that administration of anti-CD4 will prevent the negative selection of potentially self-reactive thymocytes,[14,15] suggesting a role for CD4 in the negative selection process. Anti-CD2 has been reported to not affect thymic maturation or positive selection.[16,17]

LFA-1 is also potentially important in thymic selection. Both LFA-1 and ICAM-1 are expressed in the thymus. A

Received 31 December 1993; revised 17 February 1994; accepted 19 February 1994.

Correspondence: Dr B. Richardson, R4540 Kresge, 1, Ann Arbor, MI 48109–0531, U.S.A.

majority of thymocytes expresses LFA-1 at levels slightly less than peripheral T cells,[18] and expression is relatively constant among thymocyte subsets.[19] ICAM-1 is expressed at high levels on medullary dendritic/macrophage cells and thymocytes, but only on dendritic and epithelial cells in the cortex[20] where selection takes place.[11] In contrast, thymic ICAM-2 expression appears to be confined to vascular endothelium,[20] and presumably contributes little to thymocyte–epithelial cell binding.

The presence of LFA-1 and ICAM-1 in the thymic cortex suggests that they may participate in thymic maturation. In support of this, LFA-1 has been shown to mediate binding of activated thymocytes to thymic epithelial cells,[21] and anti-LFA-1 added for 3 or more days to thymic organ cultures inhibits development of $CD4^+$ $CD8^+$ thymocytes.[19] These experiments support the concept that LFA-1 is important in thymocyte binding to epithelial cells, and raise the possibility that LFA-1–ICAM-1 interactions allow maturation to the $CD4^+$ $CD8^+$ stage.

At the $CD4^+$ $CD8^+$ stage, engagement of the TcR with specific mAb can result in apoptotic thymocyte death,[22] suggesting a mechanism by which high-affinity TcR–MHC interactions may result in negative selection. In this model, high-affinity TcR–MHC interactions would result in thymocyte apoptosis, while cells expressing lower affinity TcR would not be activated, and would survive. Since LFA-1 interactions support TcR-mediated activation in mature T cells, it is possible LFA-1 interactions also contribute to TcR-mediated negative selection in thymocytes.

The experiments described in this report test the hypothesis that LFA-1 is involved in the negative selection of thymocytes. The system used is one recently described by Kappler *et al.*[23,24] These authors demonstrated that T-cell receptors utilizing the Vβ17a gene segment bind MHC-encoded I-E molecules with high affinity. C57Br mice, which express I-E molecules, possess the gene encoding the Vβ17a allele, but lack $V\beta17a^+$ mature T cells. Immature, but not mature, C57Br thymocytes express $V\beta17a^+$ receptors, implying that $V\beta17a^+$ immature thymocytes are deleted in the thymus.[23] We hypothesized that if LFA-1 contributes to $V\beta17a^+$–I-E interactions during negative selection, inhibiting LFA-1 function should prevent activation of $V\beta17a^+$ thymocytes, preventing apoptotic death and resulting in the release of $V\beta17a^+$ T cells into the peripheral circulation. To test this, neonatal C57Br mice were injected with anti-LFA-1, and the mice examined for peripheral expression of $V\beta17a^+$ cells, using approaches previously used to determine the role of Ia and CD4 molecules in thymic selection.[11–15] The results suggest that LFA-1 also participates in negative selection.

MATERIALS AND METHODS

Mice

C57Br and SWR mice were purchased from Jackson Laboratories (Bar Harbor, ME), and housed in animal quarters supervised by the Unit for Laboratory Animal Medicine at the University of Michigan, Ann Arbor, MI.

Antibodies

KJ23a, which binds Vβ17a,[23] and KJ25, which binds Vβ3,[25] were kindly donated by Dr John Kappler. Anti-CD11a (M17/4.2)[26] and anti-CD18 (M18/2.a.8),[26] both rat IgG mAb,[26,27] were obtained from the American Type Culture Collection (Rockville, MD). The anti-CD11a mAb inhibits murine T-cell activation, while the anti-CD18 does not.[26,27] All hybridoma lines were grown according to instructions provided by the supplier, and for use were purified by affinity chromatography on protein G–Sepharose, then quantified by ELISA as described elsewhere.[28] Goat anti-mouse (GAM) Ig–RD1 was purchased from Sigma Chemical Co. (St Louis, MO). Anti-Thy-1.2–FITC, anti-CD4–FITC and anti-CD8–FITC were purchased from Coulter (Hialeah, FL). Control antibodies included an IgG anti-tubulin mAb and the murine IgG myeloma protein MOPC 21, both purchased from Sigma.

Flow cytometric analysis

To determine Vβ expression, splenocytes were incubated with KJ23a or KJ25, washed, then stained with GAM Ig–RD1. Unbound combining sites were saturated with mouse ascites, then the cells were stained with a FITC-conjugated T-cell specific antibody such as anti-Thy-1.2, anti-CD4 or anti-CD8.[9] The cells were analysed for two-colour expression on a Coulter EPICS C or ELITE flow cytometer in list mode (Hialeah, FL) and at least 50,000 cells were analysed for each histogram. Since C57Br T cells do not express Vβ17a, positive controls for Vβ17a expression included SWR splenocytes. Using this staining technique, SWR splenocytes expressed $V\beta17a^+$ on approximately 13·6 ± 4·8% of $Thy\text{-}1.2^+$ cells (mean ± SEM of five determinations), close to the 15·1% reported by Kappler *et al.*[23] Negative staining controls included isotype-matched myeloma proteins in place of KJ23a or KJ25, as well as RD1- and FITC-conjugated mouse Ig. One-colour staining of cells such as thymocytes or splenocytes was performed as previously described.[9]

Statistical analysis

The difference between means was analysed using Student's *t*-test.

RESULTS

Titration of anti-CD11a on thymocytes

Initial experiments determined the amount of anti-CD11a required to saturate thymic binding sites. Varying amounts of purified anti-CD11a or an identical amount of an IgG myeloma protein were injected i.p. into neonatal (<4 days old) C57Br mice. Four hours later the mice were killed, the thymi removed, and thymocytes stained with GAM–RD1 with or without added anti-CD11a. Figure 1a shows the staining of thymocytes isolated from a mouse receiving 150 μg of the control antibody. No significant staining with GAM–RD1 alone was observed, but incubation with anti-LFA-1 followed by GAM–RD1 gave significant staining. Figure 1b shows thymocytes from a mouse that received 150 μg of anti-CD11a. Significant and equal staining with GAM–RD1 alone and anti-CD11a plus GAM–RD1 was observed, suggesting that the thymocytes were saturated with anti-CD11a Ig *in vivo* following the anti-CD11a injection.

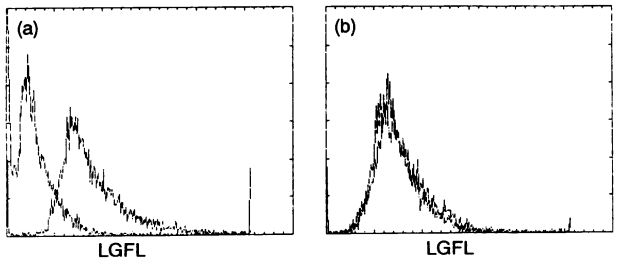

Figure 1. Binding of anti-CD11a to thymocyte CD11a. One hundred and fifty micrograms of purified anti-CD11a or an IgG myeloma protein were injected i.p. into neonatal (< 4 days old) C57Br mice. Four hours later the thymocytes were stained with GAM–RD1 with or without added anti-CD11a. (a) Thymocytes from a mouse receiving 150 μg of the control antibody. The histogram of lower fluorescence intensity represents cells stained with GAM–RD1 alone, and the higher intensity histogram represents cells stained with anti-CD11a followed by GAM–RD1. (b) Thymocytes from a mouse receiving anti-CD11a, stained as described in (a). Significant and equal staining with GAM–RD1 alone and anti-CD11a plus GAM–RD1 was seen, indicating that the thymocytes were saturated with anti-CD11a Ig *in vivo* following the anti-CD11a injection. Note that in this figure the Thy-1$^-$ RD1$^+$ population represents splenic B cells.

Role of LFA-1 in negative selection

Expression of Vβ3 and Vβ17a on Thy-1$^+$ C57Br splenocytes was then determined. Figure 2 compares Vβ3 and Vβ17a expression in normal 3-week-old C57Br mice. Vβ3$^+$ cells were readily detected, and there was no significant Vβ17a expression. In five repeats of this experiment, 7 ± 3% of Thy-1$^+$ cells expressed Vβ3, while Vβ17a expression greater than the negative staining controls was not detectable ($\leqslant$2%). These results (7·2 ± 0·4% for Vβ3, 0·1 ± 0·0% for Vβ17a) are similar to those obtained by others.[23,25]

We next asked whether neonatal injection with anti-CD11a altered peripheral expression of Vβ3 or Vβ17a. Neonatal (< 4 days old) C57Br mice were injected i.p. with 150 μg anti-CD11a, a biologically inactive anti-CD18, or a control IgG myeloma protein, and splenic Vβ3 and Vβ17a expression examined at 1, 2 and 3 weeks after injection. Controls included uninjected mice. A time frame of 1–3 weeks was chosen because

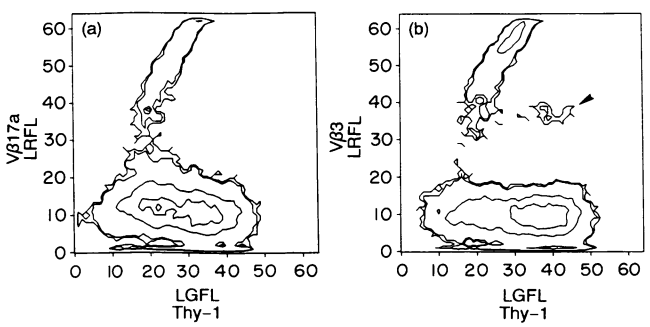

Figure 2. Expression of Vβ17a and Vβ3 on C57Br splenic T cells. Splenocytes from 3-week-old C57Br mice were stained with (a) anti-Vβ17a and GAM–RD1 followed by anti-Thy-1.2–FITC, or (b) anti-Vβ3 and GAM–RD1 followed by anti-Thy-1.2–FITC, then analysed by flow cytometry. The arrow in (b) indicates cells binding Thy-1.2 and anti-Vβ3. The relatively large Thy-1$^-$ population binding GAM–RD1 represents Ig$^+$ splenic B cells.

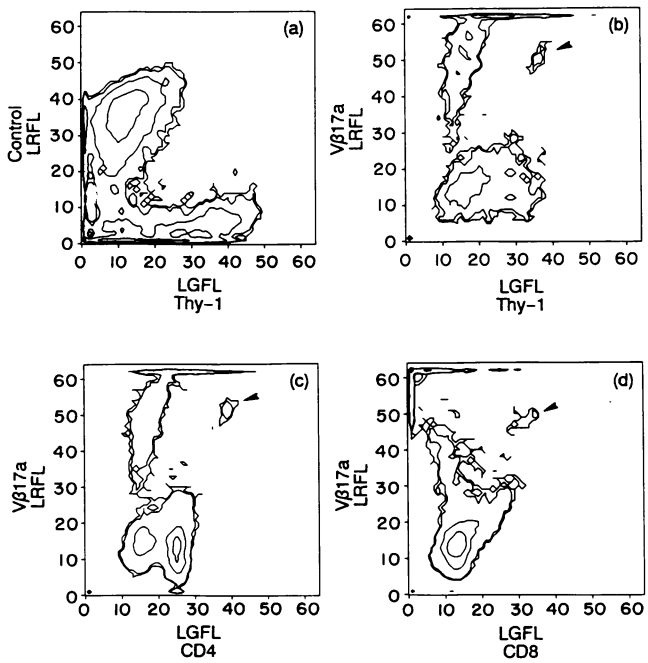

Figure 3. Neonatal C57Br mice injected with anti-CD11a express Vβ17a on splenic T cells. Neonatal C57Br mice were injected with 150 μg of purified anti-CD11a, and 3 weeks later splenocytes were stained with (a) a control IgG mAb and GAM–RD1 followed by anti-Thy-1.2–FITC; (b) anti-Vβ17a and GAM–RD1 followed by anti-Thy-1.2–FITC; (c) anti-Vβ17a and GAM–RD1 followed by anti-CD4–FITC; and (d) anti-Vβ17a and GAM–RD1 followed by anti-CD8–FITC. The arrows in b, c, and d indicate T cells binding anti-Vβ17a. As in Fig. 2, the Thy-1$^-$ GAM–RD1$^+$ population represents B cells.

of reports demonstrating that at least 2 weeks are required for thymocyte maturation and release.[30] KJ23a$^+$ splenocytes were found only at week 3, and only in mice receiving anti-CD11a. Figure 3 shows representative histograms of splenocytes from mice injected with anti-CD11a, then stained with KJ23a. Figure 3a shows the pattern observed using Thy-1–FITC and a control IgG anti-tubulin mAb and GAM–RD1. Figure 3b shows the pattern observed using KJ23a instead of the control mAb, and a distinct subset expressing Thy-1 and Vβ17a was observed. Overall, 12·2% ± 3·4% (mean ± SEM, n = 5) of splenocytes from mice receiving anti-CD11a co-expressed Vβ17a and Thy-1.2, while controls demonstrated that 1·7% ± 0·9% of splenocytes from the same mice reacted with the control of mAb (P < 0·02). Staining with the control mAb was not significantly greater than 0 (P > 0·05 by univarate t-test). In contrast, no significant changes were noted in Vβ3 expression, with 7·25 ± 1·20% (mean ± SEM, n = 6) of splenocytes from mice receiving control injections and 5·74 ± 0·68% (n = 5) of mice receiving anti-CD11a expressing Vβ3, respectively. No difference in Vβ17a or Vβ3 expression was observed between mice receiving myeloma protein injections (n = 6), anti-CD18 injections (n = 6) and uninjected C57Br mice. Also, no significant changes in the total number of splenocytes, Thy-1$^+$ splenocytes, or CD4$^+$ or CD8$^+$ splenocytes, were observed between anti-CD11a treated and control mice.

The subset distribution of the KJ23a$^+$ cells was also determined. Figure 3c shows splenocytes from a C57Br mouse

receiving anti-CD11a, and the splenocytes stained with KJ23a$^+$ GAM–RD1 and anti-CD4–FITC, while Fig. 3d shows the same cells stained with KJ23a$^+$ GAM–RD1 and anti-CD8–FITC. The CD4$^+$ KJ23a$^+$ population represented 5·9% of total splenocytes, and the CD8$^+$ KJ23a$^+$ population was 1·9% of total splenocytes relative to staining controls. Two repeats of this experiment confirmed these results. This distribution (CD4/CD8 ratio = 3·1) was essentially identical to the distribution of total CD4 and CD8 populations in these mice (ratio = 3·2).

DISCUSSION

These results demonstrate that injecting neonatal C57Br mice with sufficient amounts of mAb to CD11a to saturate thymic binding sites results in the appearance of Vβ17a$^+$ T cells in the spleen. Control injections using an IgG myeloma protein and an isotype-matched non-inhibitory anti-CD18 mAb did not alter splenic Vβ17a expression, indicating that the effect was specific for the anti-CD11a mAb. Since the Vβ17a TcR allele is normally deleted intrathymically in C57Br mice,[23] our results suggest that anti-CD11a mAb can inhibit the negative selection step of thymic maturation in this strain. No effect was observed on Vβ3 expression or overall splenic T-cell populations, suggesting that the effect was relatively specific for at least this deleted TcR allele. Given the relative specificity of the effect of the anti-LFA-1 treatment, it is reasonable to propose that inhibiting the LFA-1 function in the thymus resulted in the release of Vβ17a$^+$ T cells into the periphery. This supports the hypothesis that LFA-1 could participate in the intrathymic deletion of Vβ17a$^+$ T cells.

The identification of a potentially autoreactive T-cell subset in the peripheral circulation raises the possibility that the cells could induce autoimmunity. We and others have reported that CD4$^+$ T cells responding to Ia determinants *in vivo* can induce a lupus-like disease with the relatively rapid appearance of glomerulonephritis and autoantibodies,[28,31] suggesting that the Vβ17a$^+$ cells, also specific for self-MHC determinants in C57Br mice, could trigger a similar disease. However, urinalyses of C57Br mice injected with anti-CD11a showed no evidence for haematuria or proteinuria (B. C. Richardson, unpublished data). It is possible that the cells were tolerized and ultimately deleted in the periphery by as yet unidentified mechanisms, as has been reported by others studying similar systems.[32] Alternatively, the cells may have induced a more subtle form of autoimmunity not detected by urinalysis.

Our results complement those reported by Fine and Kruisbeek.[19] These authors reported that saturation of thymic organ cultures with anti-LFA-1 or anti-ICAM-1 for 3–4 days decreased thymic cellularity by 50% and total numbers of CD4$^+$ CD8$^+$ cells by 65–90%, while the number of immature CD4$^-$ CD8$^-$ cells did not change. This suggests that LFA-1 and ICAM-1 play an important role in the generation or maintenance of double-positive thymocytes. Since anti-LFA-1 and anti-ICAM-1 inhibit T-cell activation, it is possible that saturating amounts of these antibodies inhibit thymocyte activation during positive selection, thereby preventing maturation of the immature thymocytes, analogous to thymocytes failing to recognize self-MHC during positive selection. These authors, however, did not examine the effects of anti-LFA-1 on negative selection. Our results suggest that LFA-1 plays a similar role in negative selection as well, presumably by preventing thymocyte activation by signals which normally would activate the cell, resulting in apoptosis.

An alternative theory of thymic selection has been proposed which is independent of the LFA-1-dependent affinity considerations discussed above. In this model, cortical epithelial cells express unique proteins participating in the adherence of developing thymocytes and contributing to positive selection.[33] However, more recent reports from the same authors demonstrate that fibroblasts transfected with genes encoding the appropriate Ia proteins will also mediate positive selection.[34] This argues against the hypothesis of unique molecules on cortical epithelial cells as a mechanism for positive selection, and thus does not rule out a role for LFA-1.

The hypothesis that LFA-1 contributes to both positive and negative selection suggests a novel mechanism which could ultimately result in the selection of TcR with intermediate affinity for self-MHC. A major unanswered question is why high and intermediate affinity TcR will transmit a signal to the nucleus during the positive selection step, while only high-affinity TcR transmit activating signals during negative selection. LFA-1 could be a molecule responsible for differential effects. The avidity of LFA-1 for its ligand can be modified by the cell.[35] Phosphorylation of the LFA-1 β-chain (CD18) correlates with increased avidity for ICAM.[36] Since LFA-1 has been implicated in stabilizing TcR–Ia interactions, the fact that the avidity of LFA-1 for its ligand can be modified by the T-cell suggests a mechanism by which the same TcR could bind to an antigen-presenting cell through a high-affinity interaction during positive selection, and through a low-affinity interaction during negative selection. The high-affinity form of LFA-1 could stabilize intermediate-avidity TcR during positive selection, permitting maturation, and the low-affinity form provide less stabilization during negative selection. With less stabilization, only high-avidity receptors would transmit activating signals, resulting in apoptotic death of high-affinity cells. Such a mechanism could contribute to thymic selection, and is consistent with the known functions of LFA-1.

ACKNOWLEDGMENTS

These studies were supported by UPS grants AI25526 and AR20557, and a Veterans Administration Merit Review Grant. The authors would like to thank Ms Kristine Weber for performing the flow cytometric analyses, Ms Beverly Saunders for expert secretarial assistance, Dr David Fox for reviewing the manuscript, and Dr John Kappler for his generous contribution of anti-Vβ17a and anti-Vβ3 monoclonal antibodies.

REFERENCES

1. Davis M.M. & Bjorkman P.J. (1988) T-cell antigen receptor genes and T-cell recognition. *Nature*, **334**, 395.
2. Singer S.J. (1992) Intercellular communication and cell–cell adhesion. *Science*, **255**, 1671.
3. Doyle C. & Strominger J.L. (1987) Interaction between CD4 and class II MHC molecules mediates cell adhesion. *Nature*, **330**, 256.
4. Springer T.A. (1990) Adhesion receptors of the immune system. *Nature*, **346**, 425.

5. Dustin M.L., Sanders M.E., Shaw S. & Springer T.A. (1987) Purified lymphocyte function-associated antigen 3 binds to CD2 and mediates T lymphocyte adhesion. *J. exp. Med.* **165,** 677.
6. Marlin S.D. & Springer T.A. (1987) Purified intercellular adhesion molecule-1 (ICAM-1) is a ligand for lymphocyte function-associated antigen 1 (LFA-1). *Cell,* **51,** 813.
7. Altmann D.M., Hogg N., Trowsdale J. & Wilkinson D. (1989) Cotransfection of ICAM-1 and HLA-DR reconstitutes human antigen-presenting cell function in mouse L cells. *Nature,* **338,** 512.
8. Regnier-Vigouroux A., Blanc D., Pont S., Marchetto S. & Pierres M. (1986) Accessory molecules and T cell activation I. Antigen receptor avidity differentially influences T cell sensitivity to inhibition by monoclonal antibodies to LFA-1 and L3T4. *Eur. J. Immunol.* **16,** 1385.
9. Richardson B.C., Strahler J.R., Pivirotto T.S., Quddus J., Bayliss G.E., Gross L.A., O'Rourke K.S., Powers D., Hanash S.M. & Johnson M.A. (1992) Phenotypic and functional similarities between 5-azacytidine-treated T cells and a T cell subset in patients with active systemic lupus erythematosus. *Arthritis Rheum.* **35,** 647.
10. Lakey E.K., Margoliash E., Fitch F.W. & Pierce S.K. (1986) Role of L3T4 and Ia in the heteroclitic response of T cells to cytochrome c1. *J. Immunol.* **136,** 3933.
11. Blackman M., Kappler J. & Marrack P. (1990) The role of the T cell receptor in positive and negative selection of developing T cells. *Science,* **248,** 1335.
12. Kruisbeek A.M., Bridges S., Carmen J., Longo D.L. & Mond J.J. (1985) *In vivo* treatment of neonatal mice with anti-I-A antibodies interferes with the development of the class I, class II, and Mls-reactive proliferating T cell subset. *J. Immunol.* **134,** 3597.
13. Marrack P., Kushnir E., Born W., McDuffie M. & Kappler J. (1988) The development of helper T cell precursors in mouse thymus. *J. Immunol.* **140,** 2508.
14. MacDonald H.R., Hengartner H. & Pedrazzini T. (1988) Intrathymic deletion of self-reactive cells prevented by neonatal anti-CD4 antibody treatment. *Nature,* **335,** 174.
15. Fowlkes B.J., Schwartz R.H. & Pardoll D.M. (1988) Deletion of self-reactive thymocytes occurs at a $CD4^+8^+$ precursor stage. *Nature,* **334,** 620.
16. Yagita H., Asakawa J., Tansyo S., Nakamura T., Habu S. & Okumura K. (1989) Expression and function of CD2 during murine thymocyte ontogeny. *Eur. J. Immunol.* **19,** 2211.
17. Kyewski B.A., Jenkinson E.J., Kingston R., Altevogt P., Owen M.J. & Owen J.J. (1989) The effects of anti-CD2 antibodies on the differentiation of mouse thymocytes. *Eur. J. Immunol.* **19,** 951.
18. Kurzinger K., Reynolds T., Germain R.N., Davignon D., Martz E. & Springer T.A. (1981) A novel lymphocyte function-associated antigen (LFA-1): cellular distribution, quantitative expression, and structure. *J. Immunol.* **127,** 596.
19. Fine J.S. & Kruisbeek A.M. (1991) The role of LFA-1/ICAM-1 interactions during murine T lymphocyte development. *J. Immunol.* **147,** 2852.
20. de Fougerolles A.R., Stacker S.A., Schwarting R. & Springer T.A. (1991) Characterization of ICAM-2 and evidence for a third counter-receptor for LFA-1. *J. exp. Med.* **174,** 253.
21. Singer K.H., Denning S.M., Whichard L.P. & Haynes B.F. (1990) Thymocyte LFA-1 and thymic epithelial cell ICAM-1 molecules mediate binding of activated human thymocytes to thymic epithelial cells. *J. Immunol.* **144,** 2931.
22. Finkel T.H., Cambier J.C., Kubo R.T., Born W.K., Marrack P. & Kappler J.W. (1989) The thymus has two functionally distinct populations of immature $\alpha\beta^+$ T cells: one population is deleted by ligation of $\alpha\beta$ TCR. *Cell,* **58,** 1047.
23. Kappler J.W., Roehm N. & Marrack P. (1987) T cell tolerance by clonal elimination in the thymus. *Cell,* **49,** 273.
24. Marrack P., Lo D., Brinster R., Palmiter R., Burkly L., Flavell R.H. & Kappler J. (1988) The effect of thymus environment on T cell development and tolerance. *Cell,* **53,** 627.
25. Pullen A.M., Marrack P. & Kappler J.W. (1988) The T-cell repertoire is heavily influenced by tolerance to polymorphic self-antigens. *Nature,* **335,** 796.
26. Sanchez-Madrid F., Krensky A.M., Ware C.F., Robbins E., Strominger J.L., Burakoff S.J. & Springer T.A. (1982) Three distinct antigens associated with human T-lymphocyte-mediated cytolysis: LFA-1, LFA-2, and LFA-3. *Proc. natl. Acad. Sci. U.S.A.* **79,** 7489.
27. Sanchez-Madrid F., Simon P., Thimpson S. & Springer T.A. (1983) Mapping of antigenic and functional epitopes on the α- and β-subunits of two related mouse glycoproteins involved in cell interactions, LFA-1 and Mac-1. *J. exp. Med.* **158,** 586.
28. Quddus J., Johnson K.J., Gavalchin J., Amento E.P., Chrisp C., Yung R.L. & Richardson B.C. (1993) Treating activated $CD4^+$ T cells with either of two distinct DNA methyltransferase inhibitors, 5-azacytidine or procaimanide, is sufficient to induce a lupus-like disease in syngeneic mice. *J. clin. Invest.* **92,** 38.
29. Tulsiani D.R., Skudlarek M.D. & Orgebin-Crist M.C. (1989) Novel α-D-mannosidase of rat sperm plasma membranes: characterization and potential role in sperm-egg interactions. *J. Cell Biol.* **109,** 1257.
30. Egerton M., Scollay R. & Shortman K. (1990) Kinetics of mature T-cell development in the thymus. *Proc. natl. Acad. Sci. U.S.A.* **87,** 2579.
31. Rolink A.G. & Gleichmann E. (1983) Allosuppressor- and allohelper-T cells in acute and chronic graft-vs.-host (GVH) disease. III. Different Lyt subsets of donor T cells induce different pathological syndromes. *J. exp. Med.* **158,** 546.
32. Jones L.A., Chin L.T., Longo D.L. & Kruisbeek A.M. (1990) Peripheral clonal elimination of functional T cells. *Science,* **250,** 1726.
33. Marrack P. & Kappler J. (1987) The T cell receptor. *Science,* **238,** 1073.
34. Hugo P., Kappler J.W., McCormack J.E. & Marrack P. (1993) Fibroblasts can induce thymocyte positive selection *in vivo*. *Proc. natl. Acad. Sci. U.S.A.* **90,** 10335.
35. Dustin M.L. & Springer T.A. (1989) T-cell receptor cross-linking transiently stimulates adhesiveness through LFA-1. *Nature,* **341,** 619.
36. Buyon J.P., Slade S.G., Reibman J., Abramson S.B., Philips M.R., Weissmann G. & Winchester R. (1990) Constitutive and induced phosphorylation of the α- and β-chains of the CD11/CD18 leukocyte integrin family. Relationship to adhesion-dependent functions. *J. Immunol.* **144,** 191.