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ANTI-L-SELECTIN MONOCLONAL ANTIBODY TREATMENT IN MICE ENHANCES TUMOR GROWTH BY PREVENTING CTL SENSITIZATION IN PERIPHERAL LYMPH NODES DRAINING THE TUMOR AREA

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To examine the *in vivo* contribution of L-selectin in the sensitization of tumor-specific CTL, we investigated the effects of treatment with the anti-L-selectin monoclonal antibody (MAb) MEL-14 on the immune response to Moloney-murine sarcoma virus (M-MSV)–induced tumors, which exhibit spontaneous regression following generation of a strong virus-specific CTL response. Daily systemic administration of MEL-14 for 10 days to M-MSV-injected mice gave rise to larger sarcomas that persisted for a longer time, compared with those arising in control mice injected with virus only. The enhanced tumor growth could not be attributed to cytotoxic activity on leukocytes by MEL-14 since no reduction in the total cell number was detected in peripheral blood and spleen of MAb-treated mice. Evaluation of the immunological response in MAb-treated animals revealed a strong reduction in the generation of virus-specific CTL precursors (CTLp) in tumor-draining peripheral lymph nodes (PLN) 10 and 15 days after M-MSV injection, while in spleen, where lymphocyte localization is independent of L-selectin expression, CTLp generation was only delayed. By day 20, when tumors had begun to regress, the CTLp number showed a marked increase in both spleen and local PLN, where naive recirculating CTL could now enter because L-selectin was no longer down-regulated or blocked by the injected MAb. Our findings indicate that functional inactivation of L-selectin by MEL-14 treatment prevented migration of naive L-selectin$^+$ CTL through high endothelial venules (HEV) and their accumulation in PLN draining the tumor area, thereby precluding the initiation of a tumor-specific CTL response that takes place primarily at this site.

Adhesion of peripheral blood lymphocytes to the vascular endothelium is essential for their continuous recirculation from blood to lymph, recruitment to the site of inflammation and initiation of an immune reaction. This interaction is mediated by a vast array of cell membrane molecules, including members of the immunoglobulin and integrin families, selectins and molecules such as CD44, which are not precisely classified (Bevilacqua, 1993; Carlos and Harlan, 1994; Springer, 1994). In particular, L-selectin (CD62L), a carbohydrate-binding protein detected in mice by the MAb MEL-14 (Gallatin *et al.,* 1983), is involved mainly in the adhesion of T lymphocytes to the high endothelial venules (HEV) of peripheral lymph nodes (PLN); this receptor, which is shed after lymphocyte stimulation and re-expressed under resting conditions, is indispensable for mediating the extravasation of naive T lymphocytes in PLN (Bevilacqua, 1993). Indeed, administration of MEL-14 induces a profound alteration in lymphocyte traffic due to L-selectin down-regulation and loss from the cell surface and results in a severe depletion of these cells in PLNs (Mountz *et al.,* 1988; Rosato *et al.,* 1992; Bradley *et al.,* 1994; Lepault *et al.,* 1994; Hou *et al.,* 1995).

We have previously observed that injection of MAb directed against various cytokines and adhesion molecules is a valid approach for investigating the role of these molecules in immune reactions to tumor antigens (Zanovello *et al.,* 1988; Biasi *et al.,* 1991; Rosato *et al.,* 1992, 1995). To evaluate the effects of *in vivo* treatment with these antibodies, we used the Moloney-murine sarcoma virus (M-MSV)–induced tumor model. M-MSV is an acutely transforming retrovirus that gives rise to highly immunogenic tumors at the site of inoculation (Chieco-Bianchi *et al.,* 1988); these tumors undergo spontaneous regression that strictly depends on the generation of a strong T cell–mediated immune response, mediated for the most part by CTL specific for viral antigens (Collavo *et al.,* 1982).

The present study extends previous observations (Rosato *et al.,* 1992) on the role of L-selectin in the generation of an efficient CTL response against tumors arising in M-MSV-injected mice. We found that treatment with the anti-L-selectin MAb MEL-14 enhances tumor growth mainly by preventing CTL sensitization in the PLNs draining the tumor area.

MATERIAL AND METHODS

Mice, virus preparation and tumor induction

C57BL/6 (B6) mice, purchased from Charles River (Calco, Como, Italy), were used throughout; mice were 7 to 8 weeks old at the time of injection. Cell-free tumor extract containing M-MSV (*i.e.,* defective M-MSV co-pelleted with its natural helper M-MuLV and hereafter termed M-MSV) was prepared from primary sarcomas induced by serial passages in 1-week-old BALB/c mice. One hundred microliters of this extract, which had an *in vitro* M-MSV titer of 3×10^5 plaque-forming U/ml (PFU) on 3T3/FL cells, was injected i.m. in the thigh region of the mice. Sarcomas developed at the site of injection 5–7 days after inoculation and spontaneously regressed by days 10–16. Tumor growth was measured daily using calipers.

In vivo *treatment with anti-L-selectin MAb*

A rat hybridoma producing a MAb against mouse L-selectin (MAb MEL-14, IgG_{2a}) was obtained from the ATCC (Rockville, MD). The MAb was partially purified from ascites fluid by ammonium sulfate precipitation, dialyzed against PBS and assayed by a radial immunodiffusion kit (The Binding Site, Birmingham, UK). Treatment with MEL-14 (100 μg injected i.p.) was initiated on the same day as M-MSV injection and repeated each day for 10 days.

Cell cultures and CTL precursor frequency calculation

The frequency of M-MSV-specific CTL precursors (CTLp) in spleen and lymph node cell suspensions was estimated using a micro-mixed lymphocyte tumor culture assay (micro-MLTC), consisting of limiting numbers of responder cells, 3×10^4 irradiated (40 Gy) stimulator MBL-2 cells (a leukemic cell line originally induced by M-MuLV in B6 mice and maintained by continuous *in vivo* and *in vitro* passages) and 5×10^5 irradiated (20 Gy) syngeneic spleen cells. Cells were seeded in 96-well, round-bottomed microtiter plates (Costar, Cambridge, MA) in a final vol. of 200 μl of DMEM (Flow, Irvine, UK) supplemented with L-glutamine, HEPES, 2-ME, antibiotics, 10% heat-inactivated FCS (Flow) and IL-2 (20 U/ml,

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obtained from the supernatant of an EL-4 culture). After 7 days, cultures were tested for cytotoxic activity as follows: 100 μl of supernatant were removed and replaced with 2×10^3 ^{51}Cr-labeled MBL-2 target cells in 100 μl of medium. Microplates were incubated at 37°C for 4 hr and centrifuged, and 100 μl of supernatant were then removed for counting. Spontaneous release was determined in control microcultures prepared without responder cells. To estimate CTLp frequency, microcultures were scored as positive or negative; positive cultures were defined as those in which ^{51}Cr-release values exceeded mean spontaneous release values by more than 3 SD. Minimal estimates of CTLp frequencies were then calculated using the χ^2 minimization method described by Taswell (1981). Data were included only if the null hypothesis of no correlation with the single-hit Poisson model, based on the χ^2 of the fit, was rejected with $p < 0.05$.

Flow cytofluorimetric analysis

Peripheral blood mononuclear cells and spleen and lymph node suspensions from MAb-treated and untreated M-MSV-injected B6 mice were stained and then analyzed cytofluorimetrically (EPICS Elite, Coulter, Hialeah, FL). The percentage of MAb-coated cells was assessed by direct staining with a donkey $F(ab')_2$ anti-rat IgG-R-PE second step reagent (Jackson Immunoresearch, West Grove, PA), while the total percentage of MAb-positive cells was estimated after incubation with an unlabeled anti-L-selectin MAb followed by staining with the second step reagent. All incubations and washings were carried out at 4°C in PBS. Peripheral blood leukocyte percentages were derived using forward and side scatter parameters.

RESULTS

In vivo *treatment with anti-L-selectin MAb enhances M-MSV tumor growth*

The effect of repeated i.p. injections of MEL-14 in mice that received M-MSV in the thigh region was evaluated by measuring tumor size at different times after virus inoculation. As shown in Figure 1, MAb-treated mice developed larger tumors that persisted for a longer time, compared to those arising in control mice injected only with virus. A control group of M-MSV-injected mice repeatedly inoculated with an irrelevant isotype-matched rat IgG MAb showed a tumor growth pattern similar to that observed in mice receiving only the virus (data not shown).

To rule out the possibility that the effect of MEL-14 treatment might depend on its cytotoxic activity against leukocytes, we evaluated the total number of peripheral blood cells and percentages of different leukocyte populations. MAb-treated mice showed no significant reduction in the number of leukocytes compared to mice injected only with virus, and only by day 10 was the percentage of lymphocytes slightly reduced (32.7% *vs.* 77.5%); however, lymphopenia in these mice was counter-balanced by a concomitant increase in granulocytes (data not shown). Moreover, the total number of spleen cells in M-MSV-injected mice greatly increased and, depending on the time after virus inoculation, was 2- to 3-fold higher than the spleen cell number in uninjected control mice; however, spleen cellularity in mice receiving the MAb and in those receiving only the virus was similar (Fig. 2*a*). In contrast, the number of cells in the PLNs draining the tumor mass greatly increased at 10 and 15 days after virus injection in M-MSV-injected mice, while mice treated with both virus and MEL-14 possessed fewer PLN lymphocytes, comparable with values in control mice receiving no treatment (Fig. 2*b*). Only by day 20 was an increase in the PLN cellularity (approximately 4 times that of untreated control values) detected in the MEL-14-treated mice (Fig. 2*b*).

To further investigate the mechanism of reduced accumulation of lymphocytes in PLN draining the tumor area, we carried out flow-cytofluorimetric analysis of cells isolated from blood, spleen and PLN of MAb-treated and untreated mice. The lymphocytes of MEL-14-treated mice showed a strong down-modulation of L-selectin expression on day 10 and even on day 15, 5 days after the interruption of MAb administration; concomitantly, the disappearance of L-selectin expression was

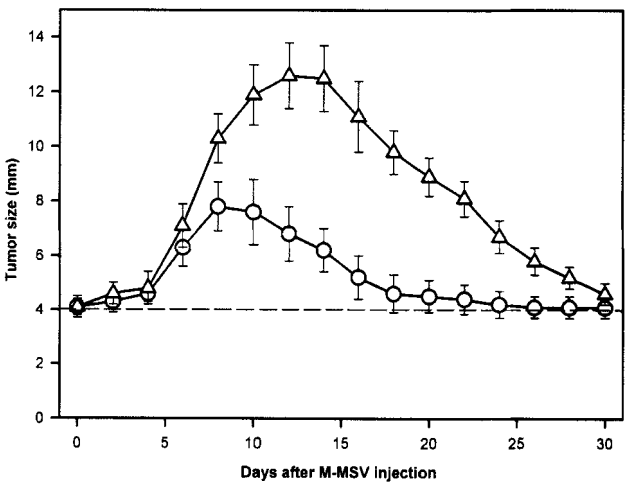

FIGURE 1 – Effect of repeated MEL-14 injection on M-MSV tumor growth. Two groups of 6 mice were injected i.m. with M-MSV in the thigh region. One group also received an i.p. injection of 100 μg of the anti-L-selectin MAb MEL-14 each day for 10 days, starting the same day as the virus inoculation. Tumor size (mm) is shown for MAb-treated (triangle) and untreated mice (circles). Broken line represents the size of the uninjected thigh.

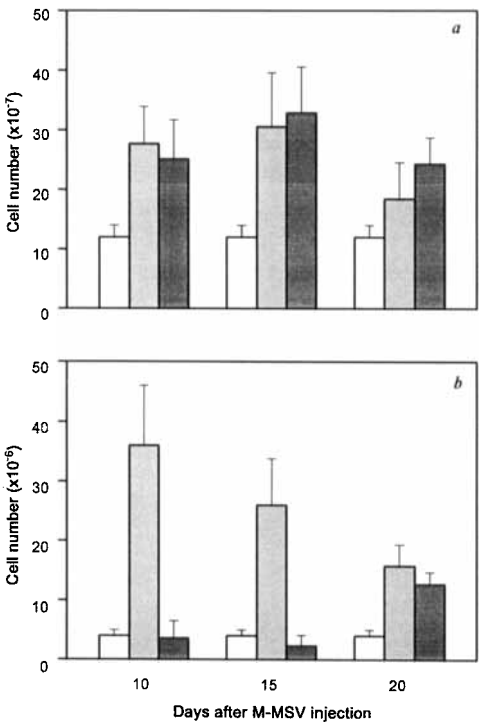

FIGURE 2 – Total cell number in spleens *(a)* and tumor-draining lymph nodes *(b)* of M-MSV-injected mice. Open bars represent control mice that received neither the virus nor the MAb, pale gray bars show control mice given M-MSV only and dark gray bars represent the number of cells in mice treated with MEL-14.

particularly evident in a large fraction of PLN cells (approx. 50% reduction compared with M-MSV-injected control mice). The cell fraction that expressed low levels of L-selectin was saturated by the MAb on day 10 and remained coated up to day 15. Subsequently, on day 20, cells re-expressed L-selectin at control levels and were uncoated by the injected MAb (data not shown).

Thus, *in vivo* administration of MEL-14 did not enhance M-MSV tumor growth through leukocyte depletion but, by down-modulating L-selectin expression, strikingly affected lymphocyte migration into PLN.

Evaluation of M-MSV-specific CTLp frequency in spleen and in lymph nodes draining tumors of MAb-treated mice

Given that M-MSV tumor regression is chiefly mediated by a strong generation of CTL directed against virus-infected tumor cells (Collavo *et al.,* 1982), we evaluated CTLp frequency and total CTLp number in the spleen and PLNs draining the tumor mass at different times after inoculation of M-MSV plus or minus MAb treatment (Tables I, II).

Ten days after M-MSV injection, mice that did not receive MEL-14 treatment showed a considerable increase in CTLp in PLNs, reaching levels similar to those detected in the whole spleen (74,980 CTLp and 85,760, respectively). The increase in CTLp depended on both the high frequency of precursors and the high cell number due to PLN cell hyperplasia (Fig. 2*b*). On the contrary, MEL-14-treated mice showed greatly reduced numbers of CTLp in PLNs, reflecting both the low frequency (Table I) and, to a greater extent, the above-mentioned hypoplasia (Fig. 2*b*). On day 15 after M-MSV injection, even mice that were not treated with MEL-14 showed a reduction in the number of M-MSV-specific CTLp in tumor-draining PLNs, while the CTLp in the spleen increased. As expected, on day 15 the CTLp remained low in the PLN of MAb-treated mice because L-selectin expression on peripheral blood cells remained reduced at that time and the few receptors present on the cell surface were covered by the injected MAb (data not shown); in contrast, CTLp showed a great increase in the spleen, as lymphocyte localization in this lymphoid organ does not depend on L-selectin expression (Table II). Finally, by day 20, total CTLp decreased even in the spleen of immune mice because by this time the tumor had already regressed (Table II). Instead, at this time point, the initiation of tumor regression in the MEL-14-treated mice was accompanied by a marked increase in CTLp number in both the spleen and local PLNs, where naive recirculating CTL were now able to accumulate due to the fact that L-selectin was no longer down-modulated and coated by MEL-14 (Table II; data not shown).

DISCUSSION

Our findings show that systemic treatment of mice with a MAb to L-selectin (MEL-14) enhances the growth of sarcomas induced by injection of M-MSV. However, such an immune-depressive effect is only transient, as the tumors undergo regression a few days after interruption of MAb administration. In this regard, we have previously observed that the age of mice at the time of inoculation of MEL-14 and the virus dose can affect tumor behavior; actually, mice injected at a younger age (5 weeks) and with a higher virus dose (10^6 PFU) developed tumors that grew rapidly and caused host death (Rosato *et al.,* 1992).

The enhancing effect of MEL-14 inoculation on tumor growth cannot be ascribed to lymphocyte depletion induced by the cytotoxic activity of the MAb employed since the total number of lymphocytes was not reduced in the peripheral blood and spleen of mice receiving repeated MAb injections. Indeed, MEL-14 administration brought about a striking down-modulation of L-selectin expression on lymphocytes, which persisted for several days after stopping MAb inoculation. Consequently, in agreement with the generally held view that L-selectin mediates the adhesion of naive lymphocytes to HEV and subsequent extravasation in PLNs, MEL-14 completely abolished the conspicuous increase in the number of lymphocytes recovered from the tumor-draining lymph nodes of M-MSV-injected mice. An accumulation of lymphocytes in PLNs draining the tumor area was observed by day 20 only, when L-selectin expression had returned to normal values. On the whole, our results agree with previous reports indicating that functional inactivation of L-selectin by treatment with MEL-14 prevents lymphocyte adhesion to HEV, thus inducing a profound alteration in PLN traffic without affecting the ability of lymphocytes to enter the spleen (Mountz *et al.,* 1988; Rosato *et al.,* 1992; Bradley *et al.,* 1994; Lepault *et al.,* 1994; Hou *et al.,* 1995). Moreover, our findings are in agreement with reports that L-selectin-deficient mice exhibit a decrease in lymphocyte infiltration and/or proliferation in PLNs draining

TABLE I – VIRUS-SPECIFIC CTLp FREQUENCY IN SPLEEN AND TUMOR-DRAINING LYMPH NODE OF M-MSV-INJECTED MICE RECEIVING ANTI-L-SELECTIN MAb TREATMENT

Treatment	Reciprocal CTLp frequency at different days after M-MSV injection[1]					
	10 days		15 days		20 days	
	Spleen	Lymph node	Spleen	Lymph node	Spleen	Lymph node
M-MSV	3,230 ± 1,350	480 ± 210	2,750 ± 1,630	3,500 ± 410	4,910 ± 2,650	3,150 ± 760
M-MSV + anti-L-selectin MAb	39,080 ± 8,950	1,960 ± 530	4,840 ± 2,410	2,850 ± 1,680	810 ± 270	450 ± 190

[1]Minimal estimates of the frequency of virus-specific CTLp were calculated by linear regression analysis of the data as reported in "Material and Methods". Each value represents the mean frequency ± SD in 6 mice.

TABLE II – TOTAL CTLp NUMBER IN SPLEEN AND TUMOR-DRAINING LYMPH NODE OF M-MSV-INJECTED MICE RECEIVING ANTI-L-SELECTIN MAb TREATMENT[1]

Treatment	Time after M-MSV injection					
	10 days		15 days		20 days	
	Spleen	Lymph node	Spleen	Lymph node	Spleen	Lymph node
M-MSV	85,760 ± 19,190	74,980 ± 20,830	111,430 ± 32,770	7,430 ± 2,230	37,670 ± 22,600	5,020 ± 3,950
M-MSV + anti-L-Selectin MAb	6,420 ± 1,690	1,840 ± 1,480	67,920 ± 15,900	810 ± 630	324,260 ± 113,630	31,370 ± 12,070

[1]Each value represents the total CTLp number ± SD in 6 mice. Total CTLp number was calculated by dividing the total cell number in the spleens and lymph nodes by the reciprocal of the frequency of M-MSV-specific CTLp.

the site of an inflammatory response (Arbonés *et al.,* 1994; Tedder *et al.,* 1995).

Regression of M-MSV-induced tumors relies on a strong T cell–mediated immune response leading to the generation of CTL which destroy virus-transformed tumor cells (Collavo *et al.,* 1982). Therefore, MEL-14-induced subversion of normal homing and extravasation of naive T lymphocytes in PLNs draining the neoplastic mass might affect their capacity to encounter tumor antigens and to be sensitized. Indeed, analysis of tumor-specific CTL generation in mice receiving MAb disclosed a low frequency of CTLp in the spleen and tumor-draining lymph nodes 10 days after M-MSV injection, the day of maximal response in control mice injected with virus only. By this time, the overall CTLp number in the spleen and tumor-draining lymph nodes of animals given only virus was similar. Therefore, the early phase of M-MSV tumor development is characterized by the generation of virus-specific CTLp, which is particularly intense in the local PLNs. The few CTLp detected in tumor-draining lymph nodes of MEL-14-treated mice might represent resident T lymphocytes, as well as CTLs that were activated at the tumor site and reached the PLN through the afferent lymphatic vessels, not requiring L-selectin to enter the PLN. In addition, although the spleen cell number was only slightly reduced in the MAb-treated mice compared to mice injected with virus alone, the total CTLp number remained low because of the low frequency of precursors. Thus, receptor modulation induced by *in vivo* MAb treatment most likely inhibited naive T-cell attachment to HEV and extravasation in PLNs, thereby preventing their sensitization by tumor cells bearing the relevant antigens.

As soon as naive CTLp undergo activation and differentiate into effector cells, they down-regulate L-selectin, thereby losing their ability to re-enter the PLN; however, by using different receptors that are up-regulated (*e.g.,* LFA-1, VLA-4, CD44), they enhance their capacity to localize in the spleen and peripheral tissues (Mobley and Dailey, 1992; Mobley *et al.,* 1995; Tripp *et al.,* 1995). Accordingly, 15 days after M-MSV injection, we observed a reduced number of tumor-specific CTLp in the lymph nodes draining the tumor area of mice injected with virus only and an increase in their spleen CTLp. However, the CTLp number in PLN of MEL-14-treated mice remained low because of L-selectin down-modulation but greatly increased in the spleen, where lymphocyte localization is independent of L-selectin expression. Similar findings were reported by Hou *et al.* (1995), who showed that CTL generation against the Sendai virus was greatly compromised in regional mediastinal lymph nodes but not in lungs or spleen. The situation appeared completely changed by day 20, when total CTLp number of mice injected with virus only decreased even in the spleen. Accordingly, studies of skin allograft rejection in mice demonstrated that reduction in antigenic stimulation brings about a concomitant decrease in a $CD8^+$L-selectin$^-$ LFA-1^+ subset that contains the effector cells having the highest lytic capacity (Mobley and Dailey, 1992). On the contrary, by this time, re-expression of L-selectin on T cells in MEL-14-treated mice allowed naive recirculating T lymphocytes to enter the PLN because tumors were large and the antigenic stimulus was still present.

On the whole, our findings indicate that the primary immune response to M-MSV takes place initially in the PLN draining the tumor area, where viral antigens or virus-infected cells localize and activate naive L-selectin$^+$ CTLs that traffic *via* HEV from blood to the PLN. By coating and down-regulating their homing receptor, MEL-14 blocks naive CTL migration in the PLN through HEV and therefore prevents CTL sensitization by tumor antigens at this site. However, MAb treatment did not alter lymphocyte localization in the spleen, where L-selectin does not appear to play a role in leukocyte trafficking, but only delayed the time required for CTLp sensitization, most likely because the contribution of effector CTLs entering the spleen after activation in local PLNs was lacking. Our results support those of others (Bradley *et al.,* 1994; Hou *et al.,* 1995), who reported that the humoral and cellular responses to KLH or Sendai virus were delayed but not blocked following MEL-14 treatment.

In conclusion, our present studies, combined with previous findings, establish that treatment with MAb directed against individual molecules involved in immune cell interactions represents a suitable experimental procedure to evaluate their *in vivo* function during the different phases of CTL reaction to viral antigens. In particular, we observed that L-selectin initiates CTL attachment to HEV and localization into the PLN. Numerous adhesion molecules and soluble factors (CD4, LFA-1, ICAM-1 and IFN-γ) are concerned with CTLp sensitization and differentiation into effector cells in peripheral lymphoid organs. Finally, LFA-1 is required for effector cell interaction with relevant target cells at the tumor site (Zanovello *et al.,* 1988; Biasi *et al.,* 1991; Rosato *et al.,* 1992, 1995). Progress in our knowledge of the function of these molecules would extend the possibility of using MAb treatment to counteract the different phases of an ongoing immunological response with great precision and without inducing irreversible damage, thus opening new perspectives in the treatment of auto-immune diseases and the prevention of allograft rejection or graft-*vs.*-host reaction.

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REFERENCES

ARBONÉS, M.L., ORD, D.C., LEY, K., RATECH, H., MAYNARD-CURRY, C., OTTEN, G., CAPON, D.J. and TEDDER, T.F., Lymphocyte homing and leukocyte rolling and migration are impaired in L-selectin-deficient mice. *Immunity,* **1,** 247–260 (1994).

BEVILACQUA, M.P., Endothelial-leukocyte adhesion molecules. *Ann. Rev. Immunol.,* **11,** 767–804 (1993).

BIASI, G., FACCHINETTI, A., PANOZZO, M., ZANOVELLO, P., CHIECO-BIANCHI, L. and COLLAVO, D., Moloney-murine-leukemia-virus tolerance in anti-CD4 monoclonal antibody treated adult mice. *J. Immunol.,* **147,** 2284–2289 (1991).

BRADLEY, L.M., WATSON, S.R. and SWAIN, S.L., Entry of naive CD4 T cells into peripheral lymph nodes requires L-selectin. *J. exp. Med.,* **180,** 2401–2406 (1994).

CARLOS, T.M. and HARLAN, J.M., Leukocyte-endothelial adhesion molecules. *Blood,* **84,** 2068–2101 (1994).

CHIECO-BIANCHI, L., COLLAVO, D. and BIASI, G., Immunologic unresponsiveness to murine-leukemia-virus antigens: mechanisms and role in tumor development. *Adv. Cancer Res.,* **51,** 277–306 (1988).

COLLAVO, D., RONCHESE, F., ZANOVELLO, P., BIASI, G. and CHIECO-BIANCHI, L., T cell tolerance in Moloney murine leukemia virus (M-MuLV) carrier mice: low cytotoxic T lymphocyte precursor frequency and absence of suppressor T cells in carrier mice with Moloney murine sarcoma (M-MSV)-induced tumors. *J. Immunol.,* **128,** 774–779 (1982).

GALLATIN, W.M., WEISSMAN, I.L. and BUTCHER, E.C., A cell-surface molecule involved in organ-specific homing of lymphocytes. *Nature (Lond.),* **304,** 30–34 (1983).

HOU, S., HYLAND, L., BRADLEY, L.M., WATSON, S.R. and DOHERTY, P.C., Subverting lymph node trafficking by treatment with the MEL-14 monoclonal antibody to L-selectin does not prevent an effective host response to Sendai virus. *J. Immunol.,* **155,** 252–258 (1995).

LEPAULT, F., GAGNERAULT, M.-C., FAVEEUW, C. and BOITARD, C., Recirculation, phenotype and functions of lymphocytes in mice treated with monoclonal antibody MEL-14. *Europ. J. Immunol.,* **24,** 3106–3112 (1994).

MOBLEY, J.L. and DAILEY, M.O., Regulation of adhesion molecule expression by CD8 T cells *in vivo. J. Immunol.,* **148,** 2348–2356 (1992).

MOBLEY, J.L., RIGBY, S.M. and DAILEY, M.O., Regulation of adhesion molecule expression by CD8 T cells *in vivo. J. Immunol.,* **153,** 5443–5452 (1995).

MOUNTZ, J.D., GAUSE, W.C., FINKELMAN, F.D. and STEINBERG, A.D., Prevention of lymphadenopathy in MRL-*Iprllpr* mice by blocking peripheral lymph-node homing with Mel-14 *in vivo. J. Immunol.,* **140,** 2943–2949 (1988).

ROSATO, A., BRONTE, V., MANDRUZZATO, S., ZAMBON, A., CALDERAZZO, F., BIASI, G., ZANOVELLO, P. and COLLAVO, D., Role of adhesion molecules in the immune reaction to M-MSV-induced tumors. *Int. J. Cancer,* Suppl. **7,** 24–27 (1992).

ROSATO, A., MANDRUZZATO, S., BRONTE, V., ZAMBON, A., MACINO, B., CALDERAZZO, F., ZANOVELLO, P. and COLLAVO, D., Role of anti-LFA-1 and anti-ICAM-1 combined MAb treatment in the rejection of tumors induced by Moloney murine sarcoma virus (M-MSV). *Int. J. Cancer,* **61,** 355–362 (1995).

SPRINGER, T.A., Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell,* **76,** 301–314 (1994).

TASWELL, C., Limiting dilution assay for the determination of immunocompetent cell frequencies. I. Data analysis. *J. Immunol.,* **126,** 1614–1620 (1981).

TEDDER, T.F., STEEBER, D.A. and PIZCUETA, P., L-selectin-deficient mice have impaired leukocyte recruitment into inflammatory sites. *J. exp. Med.,* **181,** 2259–2264 (1995).

TRIPP, R.A., HOU, S. and DOHERTY, P.C., Temporal loss of the activated L-selectin-low phenotype for virus-specific $CD8^+$ memory T cells. *J. Immunol.,* **154,** 5870–5875 (1995).

ZANOVELLO, P., VALLERANI, E., BIASI, G., LANDOLFO, S. and COLLAVO, D., Monoclonal antibody against IFN-gamma inhibits Moloney-murine-sarcoma-virus-specific cytotoxic T-lymphocyte differentiation. *J. Immunol.,* **140,** 1341–1344 (1988).