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*J Immunol* 1998; 161:641-648; ;
http://www.jimmunol.org/content/161/2/641

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Alterations in Cell Surface Carbohydrates on T Cells from Virally Infected Mice Can Distinguish Effector/Memory CD8+ T Cells from Naive Cells

Marisa Galvan,* Kaja Murali-Krishna,† Lisa Lau Ming,* Linda Baum,† and Rafi Ahmed2*‡

Glycosylation changes on surface molecules of T cells affect cell trafficking and function and may be useful in discriminating between naive, effector, and memory T cells. To analyze oligosaccharide structures on T cells activated in vivo, we examined alterations in sialic acid residues on T cells following infection of mice with lymphocytic choriomeningitis (LCMV), vaccinia virus, and vesicular stomatitis virus. We found that the majority of CD8 T cells from mice acutely infected with these viruses showed increased binding to peanut agglutinin (PNA). All of the PNA\textsuperscript{high}CD8 T cells from infected mice were CD44\textsuperscript{high}, indicating that glycosylation changes were occurring on activated T cells. There was also an increase in the PNA\textsuperscript{low}CD4 T cell population in virally infected mice. Increased PNA binding to activated CD8 T cells correlated with higher endogenous neuraminidase levels in these cells. This higher neuraminidase activity most likely contributed to the PNA\textsuperscript{high} phenotype by cleaving sialic acid residues off the core-1 O-glycans or glycoproteins destined for the cell surface. A PNA\textsuperscript{high}CD8 T cell population persisted in immune mice that had cleared the LCMV infection. When spleen cells from immune mice were sorted into PNA high and PNA low populations, 95\% of the LCMV-specific memory CD8 T cells segregated with the PNA high population. This shows that virus-specific memory CD8 T cells remain hyposialylated and can be distinguished from naive CD8 T cells based on PNA binding. Thus, PNA can be used as a marker for Ag-experienced T cells. The Journal of Immunology, 1998, 161: 641–648.

Carbohydrate structures on the surface of T cells have been found to play a role in the trafficking and homing of lymphocytes (1, 2). Carbohydrates on surface glycoproteins may be important for T cell function by modulating apoptosis and growth (3, 4). In vitro studies have shown that upon activation, the carbohydrate structures attached to glycoproteins on the surface of T cells are altered (5). Cell surface glycoproteins from activated murine and human T cells have less sialic acid residues compared with glycoproteins from resting cells (6). Furthermore, lecithins and mAbs that bind to specific carbohydrate structures demonstrate different patterns of binding to activated cells compared with resting cells (7–9). Fukuda et al. showed that upon activation with CD3 Ab or IL-2 in vitro, human T cells undergo alterations in the structure of their surface O-linked glycans (10). These alterations of surface carbohydrates during T cell activation have been observed during in vitro studies, and currently, there are no studies documenting what occurs during in vivo situations. This prompted us to examine changes in the glycosylation pattern of T cell surface glycoproteins during activation in vivo and to explore the possibility that these changes could be used to discriminate among naive, effector, or memory T cells.

The lectin peanut agglutinin (PNA)\textsuperscript{3} is known to bind T cells activated in vitro (11–14). PNA binds with the highest affinity to the disaccharide sequence Gal\textsubscript{1},3GalNAc, a sequence typically found in O-linked glycans (15). The Gal\textsubscript{1},3GalNAc disaccharide can be modified by the activity of specific enzymes. Sialyltransferase enzymes can add sialic acid residues to this structure to form tetrasaccharide SA\textsubscript{4},3Gal\textsubscript{1},3(SA\textsubscript{4},2GalNAc, while endogenous neuraminidase (sialidase) activity can remove these sialic acid residues (16). The addition of sialic acid to the Gal\textsubscript{1},3GalNAc sequence inhibits PNA binding, presumably by masking the PNA binding site. Murine T cells activated in vitro show increased PNA binding compared with resting cells, indicating loss of sialic acid from cell surface O-glycans during activation (11–14). Possible mechanisms responsible for the decrease in sialic acid residues on O-glycans on activated T cells include decreased sialylation by sialyltransferases and increased removal of sialic acid by endogenous neuraminidase (12, 14).

In this study, we have examined the changes in sialic acid residues on T cells during viral infection in vivo using the mouse model of infection with lymphocytic choriomeningitis virus (LCMV). Adult mice infected with LCMV (Armstrong strain) develop an acute infection characterized by a large expansion of activated CD8\textsuperscript{+} T cells and a potent antiviral CTL response, which mediates viral clearance within 2 wk (17, 18). The mice then develop LCMV-specific memory CD8\textsuperscript{+} T cells that persist for the life of the animal (19–21). We show in this study that activation of CD8\textsuperscript{+} T cells in vivo is accompanied by increased neuraminidase activity and decreased levels of sialic acid on surface glycoproteins. These changes are seen not only after LCMV infection, but

\textsuperscript{3} Abbreviations used in this paper: PNA, peanut agglutinin; CTLp, cytotoxic T lymphocyte precursor; int, intermediate; LCMV, lymphocytic choriomeningitis virus; 4-MU, 4-methylumbelliferyl; 4-MU-NANA, 4-methylumbelliferyl-N-acetylgalactosamine acid; PE, phycoerythrin; PFU, plaque-forming unit; VSV, vesicular stomatitis virus.
also after infection of mice with vaccinia virus and vesicular stomatitis virus (VSV). In addition, we show that virus-specific memory CD8\(^+\) T cells remain hyposialylated and can be distinguished from naive CD8\(^+\) T cells based on PNA binding.

Materials and Methods

**Mice**

C57BL/6 (H-2b) mice (6–10 wk old) used in this study were purchased from The Jackson Laboratory (Bar Harbor, MA). LCMV-immune mice were made by injecting 6- to 12-wk-old C57BL/6 (H-2b) mice with 2 \( \times 10^6 \) PFU of LCMV i.p. and were analyzed for memory CD8\(^+\) T cells greater than 30 days postinfection.

**Virus**

The Armstrong CA 1371 strain of LCMV, vaccinia virus, and VSV was used in this study. The mice were infected i.p. with 1 \( \times 10^7 \) PFU of LCMV, or with 2 \( \times 10^6 \) PFU of VSV, or with 5 \( \times 10^6 \) PFU of vaccinia virus.

**Staining reagents and flow cytometry**

FITC-conjugated anti-mouse CD8 (anti-Lyt-2.2) and phycoerythrin (PE)-conjugated anti-mouse CD4 (anti-L3T4) were purchased from Becton Dickinson (San Jose, CA). Biotin-conjugated PNA was purchased from Boehringer Mannheim (Mannheim, Germany) and used at a concentration of 0.4 mg/10^6 spleen cells in the presence or absence of 0.2 M galactose as inhibitor of specific lectin binding. PE-streptavidin or FITC-streptavidin was used in conjunction with the biotin-conjugated PNA (Caltag, South San Francisco, CA). FITC-conjugated rat anti-CD44 mAb (activation marker) was purchased from PharMingen (San Diego, CA). Mouse spleen cells were stained as described previously (19, 20).

**In vitro depletion of CD8\(^+\) T and CD4\(^+\) T cells**

To deplete CD8\(^+\) T cells, spleen cells were incubated with anti-CD8 mAb (anti-Lyt-2.2) purchased in the form of ascites fluid (Cedarlane, Hornby, Ontario, Canada), followed by treatment with low tox M rabbit complement (Cedarlane). To deplete CD4\(^+\) cells, spleen cells were treated with rat anti-mouse CD4 mAb (RL172.4) and complement. After depletion, cells were washed, counted, and assayed for neuraminidase activity.

**Neuraminidase assay**

The endogenous neuraminidase activity of whole spleen cells, CD8\(^+\)-depleted spleen cell fractions, and CD4\(^-\)-depleted spleen cell fractions was examined by a fluorometric assay, as described previously (13). Neuraminidase activity was determined by the amount of fluorogenic product 4-methylumbelliferone (4-MU) that was cleaved from its substrate 4-methylumbelliferyl-\( \alpha \)-acetilneuraminic acid (4-MU-NANA) by neuraminidase. Cells were washed in PBS, resuspended at 10^6/l in 10 mM phosphate buffer, 12 mM CaCl\(_2\) (pH 6.8), and lysed by quick freezing in a solid CO\(_2\)/ethanol bath. A total of 50 \( \mu \)l of cell homogenate was added to 25 \( \mu \)l of 0.2 mM 4-MU-NANA. A blank sample contained 0.4 mg/10^6 spleen cells in the presence or absence of 2.5 mM CaCl\(_2\) in place of cell homogenate was used to determine the nonspecific degradation of the substrate. Samples were incubated at 37°C for 1 h, and the reaction was terminated by the addition of 1 ml of 0.5 M sodium carbonate (pH 10.7). Samples were centrifuged for 10 min at 1300 \( \times \) g, and the supernatants were collected. Using a spectrofluorometer, the fluorescence was determined with emission wavelength at 430 nm and excitation at 365 nm. A standard curve was made plotting the fluorescence vs. the concentration of the 4-MU standards. The concentration of 4-MU produced in each sample was calculated by subtracting the fluorescence of the blank from the fluorescence of the sample and plotting this result on the standard curve. Activity is expressed per 10^8 spleen cells, and 1 U of activity is defined as the amount of enzyme that releases 1 mM of 4-MU per hour at 37°C. The 4-MU and 4-MU-NANA were purchased from Fluka Biochemika (Buchs, Switzerland).

**Purification and sorting of PNA\(^{high}\)CD8\(^+\) T cells**

Before sorting by flow cytometry, spleen cells from LCMV-immune mice were enriched for CD8\(^+\) T cells using the Mouse T Cell Subset Column Kit (R & D Systems, Minneapolis, MN). This enriched CD8\(^+\) T cell population was incubated for 1 h with biotin-conjugated PNA at a concentration of 4 mg/10^7 cells in 1 ml of PBS. Cells were washed twice and stained with FITC-conjugated mouse anti-CD8 mAb (Becton Dickinson) and with streptavidin-PE (Caltag) at concentrations recommended by manufacturer. PNA-labeled cells were gated on CD8\(^+\) T cells, and these cells werester-

likely sorted into PNA\(^{high}\) (top 30% fluorescent) and PNA\(^{low}\) (bottom 30%) populations on a FACStar \( \text{pro} \) dual cell sorter (Becton Dickinson). There was no crossover between the two sorted fractions, as checked by postsort flow-cytometric analysis of each sorted population. The two populations were used in limiting dilution analysis.

**Limiting dilution assay**

The LCMV-specific CTL precursor (CTLp) number and frequency in the PNA\(^{high}\) and PNA\(^{low}\) fractions were quantitated by limiting dilution assay, as described previously (19). The method of Taswell was used to determine LCMV-specific frequency (22).

**Intracellular IFN-\( \gamma \)-stain**

Spleen cells were cultured for 5 h in 96-well flat-bottom plates at a concentration of 1 \( \times 10^7 \) cells/well in 0.2 ml complete medium supplemented with 10 U/well human IL-2 and 1 \( \mu \)l/ml Brefeldin A (Golgistop; PharMingen) either in the presence or absence of CD4 or CD8 epitope peptides (1 and 0.1 \( \mu \)g/ml, respectively). To analyze CD4 responses, peptides NP 309–329 and GP 61–80 were used (23), and for CD8 responses we used NP 396, GP 33, and GP 276 (24, 25). After 5 h of stimulation, cells were harvested, washed, and surface stained with Cyochrome-Cytoperm kit, according to manufacturer’s instructions (PharMingen). For intracellular IFN-\( \gamma \)-stain, we used FITC-conjugated rat anti-mouse IFN-\( \gamma \)-mAb (clone XM 1.2).

**Results**

In *vivo* activated CD8\(^+\) T cells show an increase in PNA binding

The CD8\(^+\) CTL activity in the spleens of LCMV-infected mice peaks at approximately day 8 postinfection. At this time, the number of activated CD8\(^+\) T cells increases as much as 10-fold. Between day 8 and 15 postinfection, there is a sharp decrease in CTL activity and the number of activated CD8\(^+\) T cells. Between day 15 and 30 postinfection, the number of activated CD8\(^+\) cells continues to fall, eventually reaching homeostatic levels. LCMV-specific memory CD8\(^+\) T cells develop and are maintained at a stable frequency for the life of the mouse (19–21).

To detect changes in the sialylation state of T cells activated in vivo, we examined the ability of T cells harvested from the spleens of LCMV-infected mice at days 0, 5, 8, 15, and 30 postinfection to bind PNA (Fig. 1A). CD8\(^+\) T cells from uninfected mice (day 0) showed low levels of PNA binding (PNA\(^{low}\)). By day 5 postinfection, the activated CD8\(^+\) T cell population begins to expand in LCMV-infected mice, there was an increase in the number of PNA\(^{high}\)CD8\(^+\) T cells, and by day 8, the PNA\(^{high}\) population had expanded to comprise 29% of the spleen population. The percentage of PNA\(^{high}\)CD8\(^+\) T cells declined between days 8 and 30, corresponding with the decrease in the number of activated CD8\(^+\) cells after an acute LCMV infection. However, at day 30, a small PNA\(^{high}\) population appeared to persist when compared with naive mice (8% of splenocytes from immune mice were PNA\(^{high}\), compared with 1% of splenocytes from naive mice). Figure 2 shows the total number of PNA\(^{high}\)CD8\(^+\) T cells per spleen at various time points (days 5, 8, 15, 30, >60) after LCMV infection. Notice that at the peak of infection (day 8) there was a greater than 15-fold increase in the number of PNA\(^{high}\)CD8\(^+\) T cells.

To determine whether PNA was preferentially binding to activated T cells, CD8\(^+\) T cells were purified and double stained with PNA and CD44 (an activation marker for T cells). As shown in Figure 1B, there was a complete correlation with activation (CD44\(^{high}\)) and PNA\(^{high}\) binding. At 8 days postinfection, nearly all CD8\(^+\) T cells in the spleens of LCMV-infected mice were CD44\(^{high}\), and all of these cells showed increased PNA binding. This is in contrast to CD8\(^+\) T cells from uninfected mice, in which the majority of the cells were CD44\(^{low}\) and PNA\(^{low}\). Note that even in normal mice the population of CD8\(^+\) T cells that was
CD44 high showed increased binding to PNA. However, the levels of both CD44 and PNA were lower in this population than that seen in CD8\(^+\) T cells from LCMV-infected mice.

Alteration in cell surface carbohydrates on activated CD8\(^+\) T cells is a common feature of viral infection

To determine whether the increase in PNA binding on activated CD8\(^+\) T cells was specific to LCMV infection or was a general phenomenon in viral infections, we examined the pattern of PNA binding to CD8\(^+\) T cells in mice acutely infected with vaccinia virus or VSV. At 8 days postinfection, splenic CD8\(^+\) T cells from virally infected mice were stained with PNA and anti-CD8 Ab and analyzed by flow cytometry (Fig. 3). Compared with uninfected mice at day 0, the vaccinia virus-infected mice showed a shift in the CD8\(^+\) population from PNA low to PNA high. This shift was also seen in VSV-infected mice, although the magnitude of the shift was less than that seen with vaccinia virus infection. In both cases, as observed in LCMV infection, there was an increase in PNA binding to CD8\(^+\) T cells activated during viral infection. Thus, increased PNA binding, i.e., decreased cell surface sialylation of activated CD8\(^+\) T cells, was not restricted to LCMV infection, but appeared to be a general phenomenon in viral infections.

CD8\(^+\) T cells activated in vivo express high levels of neuraminidase activity

As mentioned previously, PNA binds with high affinity to the disaccharide Galβ1,3GalNAc, which is found on O-linked oligosaccharides. Sialic acid can be added to this structure, and the presence of sialic acid masks the PNA binding sites. One mechanism that could account for the changes in PNA binding involves the removal of sialic acid by endogenous neuraminidase activity. Higher levels of neuraminidase activity could increase the number of exposed PNA binding sites on cell surface glycoproteins. Therefore, we examined whether T cells from LCMV-infected mice had an increased endogenous neuraminidase activity. Using a fluorometric assay, we analyzed the endogenous neuraminidase activity in spleen T cells from uninfected and LCMV-infected mice. As shown in Figure 4A, total spleen cells from day 8 LCMV-infected mice had approximately threefold greater neuraminidase activity than spleen cells from uninfected mice. Furthermore, the increase in neuraminidase activity was found almost exclusively in CD8\(^+\) T cells (Fig. 4B). In samples in which the CD8\(^+\) T cells were depleted by anti-CD8 mAb and complement treatment, there was approximately a 2.5-fold decrease in neuraminidase activity. If CD4\(^+\) T cells were depleted, there was only a slight decrease in activity. These experiments suggest that increased endogenous neuraminidase activity in CD8\(^+\) T cells exposed cell surface ligands for PNA by removing inhibitory sialic acid residues.
Upon examination of the kinetics of PNA binding to CD8
1 T cells during LCMV infection, a PNA high CD8
1 T cell population appeared to persist at day 30 and beyond (greater than day 60). We investigated whether this population contained LCMV-specific memory CD8
1 T cells. We purified CD8
1 spleen cells from LCMV-immune mice (45 days postinfection), as described in Materials and Methods. These mice have cleared LCMV infection and developed anti-LCMV memory CD8
1 T cells. The CD8
1 cells were double stained with PNA and anti-CD8 Ab and sorted by flow cytometry (Fig. 5A). We isolated two cell populations, PNAhigh and PNAlow. Each population was analyzed by limiting dilution to determine the LCMV-specific CTLp frequency (Fig. 5A). Within the PNAhigh population, the LCMV-specific frequency was high (1/10
2). In contrast, the LCMV-specific CTLp frequency in the PNA low population was low (1/9.3 10
3). Thus, virtually all of the LCMV-specific memory CTLp segregated with the PNAhigh population (99% in the PNA high population and 1% in...
the PNA\textsuperscript{low} population). To further examine the specificity of PNA\textsuperscript{high} and PNA\textsuperscript{low} CD8 as a function of time, several time points after infection were tested, and majority of virus-specific CD8 were PNA\textsuperscript{high} at all of the time points (Fig. 5B). These results show that the changes in the sialylation state of O-glycans on T cell surface glycoproteins that occur during activation in vivo are maintained on memory cells. Thus, PNA binding can be used as a marker for memory T cells. Interestingly, this population of CD8\textsuperscript{+} memory T cells that bound PNA did so at a relatively lower level (PNA intermediate or PNA\textsuperscript{int}) than some of the CD8\textsuperscript{+} T cells from the acute stage of LCMV infection (day 8 postinfection) (Fig. 6). These results suggest that the level of PNA binding may be useful in discriminating not only between naive and memory cells, but also between effector and memory CD8\textsuperscript{+} cells.

**Increased PNA binding of in vivo activated CD4\textsuperscript{+} T cells**

Since changes in PNA binding on CD8\textsuperscript{+} T cells from virally infected mice were detected, it was of interest to determine whether similar changes were taking place on CD4\textsuperscript{+} T cells during infection. As shown in Figure 7A, there was an increase in the number of CD4\textsuperscript{+}PNA\textsuperscript{high} spleen cells in mice acutely infected with LCMV. The number of CD4\textsuperscript{+}PNA\textsuperscript{high} T cells in the spleen in uninfected (day 0) mice was $1.3 \times 10^6$. By day 8 postinfection, this number increased by more than threefold to $4.3 \times 10^6$/spleen.
By day 30 postinfection, the number of CD4\(^{+}\)PNA\(^{high}\) T cells decreased to levels near what was observed in uninfected mice. The binding of PNA to CD4\(^{+}\)T cells from VSV- and vaccinia virus-infected mice was also examined. As was seen with LCMV infection, the PNA\(^{high}\)CD4\(^{+}\) T cell population increased by fivefold in VSV-infected mice and eightfold in the vaccinia-infected mice (Fig. 7B). At day 8 postinfection, there were 6.7 \(\times\) 10\(^6\) and 11 \(\times\) 10\(^6\) PNA\(^{high}\)CD4\(^{+}\) T cells in the spleens of VSV- and vaccinia-infected mice. These data showed that changes also occurred in the sialylation state of surface glycoproteins on mouse CD4\(^{+}\) T cells during viral infection. To examine whether the PNA\(^{high}\)CD4\(^{+}\) T cells contained virus-specific cells, spleen cells from LCMV-infected mice (day 8) were cultured for 5 h with CD4 T cell-specific LCMV peptides (23), followed by intracellular IFN-\(\gamma\) stain. The responses of CD4 T cells in PNA\(^{high}\) and PNA\(^{low}\) CD4 cells were analyzed. As shown in Figure 8, 7% of the PNA\(^{high}\)CD4 cells were LCMV specific, whereas only 0.4% of PNA\(^{low}\)CD4 cells made IFN-\(\gamma\) after peptide stimulation. Thus, most of the (93%) virus-specific CD4 T cells were PNA\(^{high}\). Note that neither PNA\(^{high}\) nor PNA\(^{low}\)CD4 T cells from naive mice responded to the peptide stimulation. Hence, these experiments demonstrate that PNA can also act as a marker for in vivo activated Ag-specific CD4 T cells.

**Discussion**

The pattern of glycosylation on T cell surface glycoproteins changes as the cells mature in the thymus and migrate to the periphery (26). It would be useful to identify unique oligosaccharides that characterize T cell subsets, especially if these structures can be used to distinguish memory T cells and effector T cells from naive T cells. In this study, we showed that T cells activated in vivo during a viral infection bind PNA at a higher level than naive cells. These data indicate that T cell activation resulted in altered cell surface glycosylation, specifically loss of sialic acid from O-glycans. Memory CD8\(^{+}\) T cells remained PNA\(^{high}\), indicating that the decreased sialylation that occurred during activation was maintained in this population.

Spleen cells from mice infected with LCMV were stained with PNA to determine the pattern of expression of the PNA ligand. At the peak of the virus-induced activation and expansion of CD8\(^{+}\) cells and the peak of the CTL activity, the majority of the CD8\(^{+}\) population stained PNA\(^{high}\). The increase in PNA binding correlated with the activation of the CD8\(^{+}\) cells, as the PNA\(^{high}\)CD8\(^{+}\) cells were also CD44\(^{high}\). We saw a similar increase in PNA\(^{high}\)CD8\(^{+}\) cells in mice during VSV and vaccinia virus infections. These data show that the increase in PNA binding to CD8\(^{+}\) T cells is a general property of T cell activation during viral infection. Similar changes were also observed in CD4\(^{+}\) T cells.

There are two mechanisms that could account for the increase in PNA binding to activated T cells. PNA binds to the disaccharide sequence Gal\(\beta\)1,3GalNAc, which is found on O-linked...
oligosaccharides attached to glycoprotein structures on the surface of cells. Sialic acid can be added to this disaccharide structure by two sialyltransferase enzymes to form the sequence SA\(\alpha_2,3\)Gal\(\beta_1,3\)(SA\(\alpha_2,6\)GalNAc) (16). When sialic acid is present, the PNA binding site is masked. A decrease in the expression or activity of sialyltransferase enzymes in T cells may result in the expression of surface glycoproteins that are undersialylated, with more sites available to bind PNA. Alternatively, sialic acid residues could be cleaved off nascent or recycled glycoproteins by an endogenous neuraminidase (12, 27). Thus, an increase in endogenous neuraminidase activity could account for the increase in PNA binding. In this study, we showed that T cells from day 8, acutely infected mice had increased endogenous neuraminidase activity compared with CD8\(^+\) T cells from uninfected mice. This suggests that upon activation of these cells, sialic acid is cleaved off glycoprotein structures, exposing PNA binding sites.

When CD4\(^+\) T cells were depleted from spleen cell samples from day 8 LCMV-infected mice, there was only a slight decrease in neuraminidase activity compared with samples that contained CD4\(^+\) T cells. However, we did observe an increase in the CD4\(^+\)PNA\(^{\text{high}}\) T cell population in LCMV-infected mice. It is possible that due to the smaller number of activated CD4\(^+\) T cells compared with activated CD8\(^+\) T cells, the neuraminidase assay was not sensitive enough to detect changes in neuraminidase activity in the CD4\(^+\) T cell-depleted population, or it may be that the changes in cell surface carbohydrates on CD4\(^+\) T cells that we observed are caused by a different mechanism.

When CD8\(^+\) T cells from LCMV-immune mice (day 30 or greater postinfection) were stained with PNA, we saw a persistent population of PNA-binding cells. Based on limiting dilution analysis, we showed that the LCMV-specific memory CTLp were contained in this population. Where do these memory cells come from, and when do these changes associated with them develop? Memory cells could first exist as effector cells, binding PNA at a very high level. After clearance of infection, as the population of activated CD8\(^+\) cells returns to basal levels, some effector cells may down-modulate their PNA binding, and live on as memory cells. Alternatively, the PNA\(^{\text{int}}\) cells could be a population that exists separately from the PNA\(^{\text{high}}\) effectors, and after the clearance of infection, the PNA\(^{\text{int}}\) cells could live on to become memory cells while the PNA\(^{\text{high}}\) cells die.

The T cell surface glycoproteins that bind PNA during activation are currently being explored. Recently, Wu et al. showed that PNA bound to CD43, CD45, and CD8 molecules on mouse thymocytes (28). Furthermore, Casabo et al. showed that during T cell activation, O-linked sugars on the CD8\(\beta\) chain become desialylated (29). The molecules that bind PNA on activated T cells and memory cells have not been identified. Since the level of PNA binding is higher on effector CD8 T cells (day 8 postinfection) than in memory cells, it is possible that PNA staining may identify cell surface molecules that can distinguish between memory and effector cells. Furthermore, the functional importance of the alteration in sialylation needs to be examined. Carbohydrate structures attached to surface glycoproteins play a role in T cell circulation, trafficking, and adhesion (30). The glycosylation changes documented in this study could be important for trafficking and localization of memory cells and effector cells to sites of Ag presentation. Alterations in cell surface sialylation may also affect T cell recognition of APCs, or interactions with accessory molecules. For example, CD23 on B cells has been shown to bind to the Gal\(\beta_1,3\)GalNAc sequence (31). The Gal\(\beta_1,3\)GalNAc sequence can be masked by the addition of sialic acid, resulting in the inhibition of CD23 binding. Moreover, similar to what we documented in this study, changes in the glycosylation pattern on the surface of B cells have been shown to occur specifically on the
surface of Ag-specific B cells in germinal centers of mouse lymph nodes (32, 33). The changes in sialylation documented in this study may play a role in T cell signaling by acting through such molecules as CD8 and CD43 (29, 34). The dynamic modulation of T cell surface glycosylation indicates that these changes affect T cell function, in addition to allowing the phenotypic discrimination between naive and effector/memory T cells.

Acknowledgments

We thank Dr. N. Perillo and R. Concepcion for excellent technical assistance.

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