Chronic Alcohol Consumption Increases the Severity of Murine Influenza Virus Infections

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Chronic Alcohol Consumption Increases the Severity of Murine Influenza Virus Infections


Respiratory infections with both seasonal as well as potential pandemic Influenza viruses represent a significant burden on human health. Furthermore, viruses such as Influenza are increasingly recognized as important etiologic agents in community acquired pneumonia. Within the U.S. alone, ~12.9 million people are heavy drinkers and chronic abuse of alcohol is known to increase the risk and severity of community acquired pneumonia. Given the lack of knowledge regarding Influenza disease in this population, we determined the effects of chronic alcohol consumption on Influenza virus infection. Herein, we report that mice exposed to chronic ethanol have sharp increases in morbidity, mortality, and pulmonary virus titers relative to controls. These increases in influenza severity correspond with inhibited pulmonary influenza-specific CD8 T cell responses. Further, chronic ethanol consumption results in an enhanced pulmonary lesion severity, similar to that recently described for pandemic influenzas. Together, our results suggest that chronic alcohol consumption may increase the risk for severe influenza virus infections by altering the pulmonary inflammatory environment and CD8 T cell response. The Journal of Immunology, 2008, 181: 641–648.

Influenza A virus infections represent a serious challenge to human health and are increasingly recognized as an important agent in community-acquired pneumonia (CAP)1 (1, 2). In addition to primary disease, influenza infection is known to predispose individuals, particularly persons with underlying medical conditions, to an increased incidence of secondary pneumonias, which in turn leads to more severe disease outcomes (3–6). A recent study of CAP hospitalizations during influenza season demonstrated that those patients with a history of prior influenza vaccination had a significantly reduced mortality compared with unvaccinated patients, which portrays the increasingly recognized role of influenza in CAP (7). Individuals chronically abusing alcohol have a predisposition for CAP and are more prone to severe disease outcomes (8, 9). Although bacterial pneumonias are among the best-studied examples of CAP in individuals with chronic alcohol abuse, resulting in a 2- to 7-fold greater incidence in mortality and morbidity compared with non-alcoholic pneumonia patients, there is a lack of studies investigating influenza disease and chronic alcohol (10–14). Within the U.S. alone, ~65% of the population has consumed alcohol within the last year. Among these individuals, ~16% are classified as heavy drinkers with another ~21% classified as moderate drinkers (15). Given this prevalence of alcohol consumption and the current threat of both seasonal, epidemic, and potential pandemic influenza (i.e., H5N1 Avian influenza) (3–5), a better understanding of the lesions within pulmonary adaptive immune responses among heavy alcohol-consuming populations could aid in designing strategies to boost immunity in these individuals to this important human pathogen.

Although the role of chronic alcohol consumption in increasing the severity of respiratory bacterial infections is well recognized, its effects on viral infections such as influenza virus is lacking. It is well accepted that chronic ethanol ingestion is immuno-suppressive and exerts general inhibitory effects on the adaptive immune response, particularly within the CD8 T cell compartment (9, 16–23). However these studies have largely focused upon examining the immunosuppression of cell-mediated immunity in the spleen, skin, and liver. Importantly, for many pathogens, immune responses may be tissue specific and those in the respiratory and interstitial mucosa are distinct from that generated following i.v., i.p., or s.c. exposures. Therefore, what progression of lesions is found in the respiratory adaptive immune response within alcoholics and the consequences of these lesions, particularly during virus infections, remains in question.

Materials and Methods

Mice

Six to seven week old female C57BL/6 and BALB/c mice were obtained from the National Cancer Institute (Frederick, MD). All mice were housed and maintained in the animal care facility at the University of Iowa. All experiments were performed in accordance with regulatory standards and guidelines and were approved by the institutional (University of Iowa) Animal Care and Use Committee.

EtOH administration

After 1 wk of acclimation, mice from the same purchase lot were separated into two groups of equal size, with one group to be phased on to EtOH (pharmaceutical grade). EtOH was provided in the drinking water at 10% (weight/volume) for 2 days, 15% for 5 days, and 20% for C57BL/6 or 18% for BALB/c mice for 4–8 wk. All time points in the results are listed as the length of time at the final EtOH concentration. Mice were studied at 4- and 8-wk time points. The mice were provided laboratory chow ad libitum in all cases, and control animals were given the same double-distilled water
as that used for mixing the EtOH solution. Mice were maintained on the same water or EtOH protocol during influenza infection.

**Influenza virus infection**

Mouse-adapted influenza A virus A/PuertoRico/8/34 (H1N1) and A/ JAPAN/305/57 (H2N2) were grown in the allantoic fluid of 10-day old embryonated chicken eggs for 2 days at 37°C, as previously described (24). Allantoic fluid was harvested and stored at −80°C. Mice were anesthetized with Isoflurane or a mineral oil mixture containing 33% Halothane (Halocarbon Products). C57BL/6 and BALB/c mice were infected intranasally with 3.155 × 10^6 pfu dose of A/PR/8/34 and a 1.985 × 10^7 pfu dose of A/JAPAN/305/57, respectively. Morbidity, as measured by weight loss, and mortality were monitored daily.

**T cell analysis of intracellular IFN-γ production**

Single-cell suspensions of lungs were cultured at 5 × 10^5 cells/well with either media, influenza peptides individually (PA234 and influenza nucleocapsid protein (NP)366 for C57BL/6 mice or HA204, HA529, and NP147 for BALB/c mice) or an influenza peptide mix for 6 h at 37°C. Cells were fixed with FACS Lysis Solution (BD Biosciences), permeabilized with Saponin (Acros Organics), and stained with rat anti-mouse CD8 (16, 17, 20). Importantly, this model results in a range of immune cell responses. The concentration of NP in homogenized influenza infected lungs was quantified using the Reed-Muench accumulative method. A hemagglutination assay was performed to determine the lung virus titer.

**Histopathology examination**

Lungs were inflated with PBS containing heparin, tied off, and removed from infected animals on days 5, 6, and 8 postinfection (p.i.) and placed in 10% formalin. After 10 days in formalin the lungs were initially assessed grossly for percentage of lung affected and then embedded into paraffin, sectioned, and examined by H&E. All gross and microscopic lesions were scored as follows: Atelectasis - 0, absent; 1, detectable; 2, mild foci; 3, multifocal areas; 4, widespread to the entire lung. Alveolar edema - 0, absent; 1, detectable; 2, mild foci; 3, moderate; 4, severe.

**Lang virus titer**

Lungs from infected mice were rapidly homogenized, snap frozen, and stored at −80°C. Subsequently serial dilutions of homogenized lungs were injected into groups of 10-day old fertile eggs and then the eggs were incubated for an additional 48 h at 35°C. A hemagglutination assay was then performed by mixing 25 μl of allantoic fluid with 25 μl of 1% chicken blood and incubating the mixture for 20 min at room temperature. Virus titer was quantified using the Reed-Muench accumulative method.

**Influenza NP ELISA**

The concentration of NP in homogenized influenza infected lungs was determined by sandwich ELISAs on days 6 and 8 p.i. The anti-NP mAb (H19-S24-4.3, capture) and biotinylated anti-NP (H16-L10-4R5, secondary) Abs were provided by Walter Gerhard (The Wistar Institute, Philadelphia, PA) and purified NP standard was affinity purified following lysis of A/PR/8/34 virions.

**Results**

**Chronic EtOH consumption increases influenza-associated morbidity, mortality, and pulmonary viral loads**

Based on the work of Meadows and colleagues (21), we have established an EtOH-in-drinking-water mouse model of chronic alcohol intake (hereafter referred to as the Meadows-Cook model) (16, 17, 20). Importantly, this model results in a range of immune and tissue lesions that are normally observed in chronic alcoholics without inducing an overt stress response (16). Likewise, experimental influenza infection of mice with mouse-adapted influenza strains offers a well-characterized model that allows careful study of the pathogenesis and immune mechanisms that allow control and elimination of the virus (24–33). Therefore, experimental influenza infection of mice on the Meadows-Cook protocol serves as a good model in which to determine how EtOH affects the immune response and severity of influenza infections. Hence, BALB/c mice were placed on chronic EtOH for 4 wk and then infected intranasally with a 0.01 LD50 dose of influenza virus and then assessed for morbidity as measured by weight loss (A) and mortality (B). One representative experiment of two (8–10 mice/group) is shown in A and B.

**FIGURE 1.** Chronic EtOH consumption increases the severity of influenza virus infections. EtOH-(circle) and water-(triangles) conditioned groups of BALB/c mice were infected with a 0.01 LD50 dose of influenza virus and then assessed for morbidity as measured by weight loss (A) and mortality (B). One representative experiment of two (8–10 mice/group) is shown in A and B.
**Chronic EtOH consumption decreases influenza-specific CD8 T cell responses**

Many studies have shown that protective immunity to primary influenza virus infection involves the clearance of infected epithelial cells by CD8+ T cells through Fas and perforin-dependent direct killing mechanisms (27, 34). These T cells first appear in the lungs around day 4 p.i. where their continued expansion and accumulation corresponds with virus clearance (24). The importance of CD8 T cells in protection from influenza infections is further highlighted by the fact that CD8+ T cells mediate resistance to and protection from lethal influenza virus infections even in the absence of B cells, CD4+ T cells, and Ab (26, 30). Therefore, given the increased pulmonary virus titers observed above, we determined whether chronic EtOH consumption altered the pulmonary influenza-specific CD8 T cell response after only 4 wk of alcohol consumption. Indeed, chronic EtOH exposure decreases the total number of CD8 T cells within the lungs by ~50% after infection (Fig. 3A). Further, this decrease in total CD8 T cells, as measured by influenza-peptide MHC tetramer analysis (Fig. 3B) or intracellular IFN-γ staining (Fig. 3C), is reflected in a corresponding reduction in the total number of influenza-specific CD8 T cells. However, when the influenza-specific response is examined as a frequency of the total CD8 T cells within the lungs (Fig. 3D and E), there is no difference between the water and EtOH groups. This result implies that both the lung environment and secondary lymphoid tissues were in a general immunocompromised state due to chronic EtOH consumption, as previously described (16, 17, 20, 35). Importantly, the compromised immune status of the chronic EtOH host resulted in a poor response and reduced total numbers of influenza-specific effector T cells. Given the role influenza-specific effector CD8 T cells play in elimination of influenza-infected cells (26, 36), the loss of these T cells suggests that pulmonary influenza-specific cytotoxicity would also be reduced, an outcome that could be responsible for the increases in influenza virus titers and Ag loads observed in these mice (Fig. 2).

**FIGURE 2.** Chronic EtOH consumption increases the magnitude and duration of influenza virus infections. EtOH-(black bars) and water-(open bars) conditioned groups of BALB/c mice were infected with a 0.01 LD_{50} dose of influenza virus and then assessed for pulmonary virus titer (A), and NP loads (B). Data are the mean ± SEM of 5–9 mice/group.

**FIGURE 3.** Chronic EtOH inhibits pulmonary Influenza-specific CD8+ T cell immune responses. Four-week water-(open bars) or EtOH-(closed bars) conditioned BALB/c mice were infected with influenza A virus. On day 8 p.i., the number of total pulmonary CD8 T cells (A) as well as the number (B and C) and percentage (D and E) of influenza-specific CD8+ T cells were determined by flow cytometry. Cells were identified by binding of influenza-specific MHC-tetramers (B and D) or by influenza-peptide (HA204, HA529, NP147, or control RSV M280) induced intracellular IFN-γ staining (C and E). One representative experiment of two (minimum of four mice/group, mice analyzed individually) is shown. Data are the mean ± SEM. *, p < 0.05.

As chronic alcoholics are from genetically diverse backgrounds and multiple strains of influenza A virus are responsible for disease, we next determined whether the observed inhibition in influenza-specific T cell immunity was unique to BALB/c mice or the mouse-adapted H2N2 strain of influenza A virus used. To this end, we infected 4-wk water and EtOH treated C57BL/6 mice with mouse-adapted A/PR/8/34 (H1N1) influenza A virus. As shown in Fig. 4, the total number of pulmonary CD8α+ T cells (Fig. 4A) and influenza-specific CD8 T cells (Fig. 4D) were reduced in EtOH mice similar to the results observed in BALB/c mice infected with the H2N2 Influenza A virus (Fig. 3, A–C). These results indicate that the effects of chronic EtOH on influenza immunity and disease are not unique to either the mouse or influenza virus strain used.

**Influenza-specific CD8 T cell responses become dysregulated during extended chronicity of EtOH consumption**

Previous studies have suggested that many of the defects EtOH has on the immune system worsen with the chronicity of EtOH exposure (16). Therefore, to determine whether the inhibition in T cell immunity observed at 4 wk of EtOH was maintained, lost, or strengthened by further EtOH exposure, we infected C57BL/6 mice that had been conditioned with EtOH for 8 wk. As shown in Fig. 4A, chronic EtOH for 4 wk reduces the total pulmonary CD8α+ T cell response to influenza by ~50%. This inhibition is further increased to ~70% by 8 wk of EtOH exposure (Fig. 4E).
These results indicate that the defects in pulmonary CD8 T cell immunity increase with the length of exposure to EtOH.

Next, we determined whether the chronicity of EtOH altered the number and effector capacity of influenza-specific CD8 T cells. Similar to what occurs in BALB/c mice (Fig. 3), the C57BL/6 EtOH mice have substantially fewer influenza-specific CD8 T cells (Fig. 4D) than water controls. This appears to be due primarily to the loss in total pulmonary CD8α− cells in 4-wk EtOH mice (Fig. 4A), as the % of tetramer+ (Fig. 4B) or IFN-γ+ (Fig. 4C) cells among pulmonary CD8α− cells is not significantly different between the water and EtOH mice. Interestingly, however, the influenza specific CD8 T cell response in 8-wk EtOH mice shows a defect in addition to the above-described reduction in the total number of cells. Whereas the percentage of tetramer+ influenza-specific T cells among total CD8 T cells is equal between the water and EtOH groups (Fig. 4F), the EtOH, but not water, 8-wk T cells are dysregulated and do not make IFN-γ in response to stimulation with influenza virus peptides (Fig. 4G). This dysregulation results in a significant loss of IFN-γ+ influenza-specific T cells from the lungs (Fig. 4D). Interestingly, however when the 8-wk EtOH T cells were stimulated in vitro with PMA/ionomycin, instead of influenza virus peptides, equivalent percentages of CD8 T cells produced IFN-γ+ (Fig. 5). Because PMA and ionomycin bypass the TCR/CD3 complex to deliver stimulatory signals to T cells by activating protein kinase C (PKC) and raising cytoplasmic calcium concentrations, these results suggest that a defect in signaling upstream of PKC and Ca2+ flux leads to the dysregulated ability 8-wk EtOH influenza-specific effector CD8 T cells to produce IFNγ.

Together our results indicate that the influenza-specific T cell response develops progressively greater defects both in the number of cells available as well as in their effector phenotype as the length of exposure to EtOH increases. Given the integral role CD8 T cells play in the elimination of virally infected cells during primary influenza infections (24, 27, 30), these results, when combined with those in Figs. 1 and 2, suggest that the increased severity of influenza virus infections following chronic EtOH consumption might in part be related to the decrease in influenza-specific T cell immunity. Similar results showing increased morbidity and mortality as well as inhibited CD8 T cell and influenza-specific CD8 T cells and function relative to age-matched water controls were observed when BALB/c were examined following 8 wks of chronic EtOH ingestion (data not shown).
Chronic EtOH consumption is associated with neutrophilic and severely edematous lungs during influenza infections

Recent studies on pandemic (i.e., 1918 H1N1 Spanish influenza) or potential pandemic (H5N1, Avian influenza) strains have shown that in the absence of adaptive CD8 T cell responses or overwhelming innate immune responses the pulmonary pathology associated with influenza infections is significantly increased (25, 28, 37). Therefore, to determine whether the reduced numbers of influenza-specific CD8 T cells observed in chronic EtOH mice would lead to a similar increase in pulmonary disease, we infected 4- and 8-wk EtOH or water conditioned C57BL/6 with A/PR/8/34 and performed histopathological analysis. At both the gross (Fig. 6A and B) and microscopic level (Fig. 6, C–H), lungs from 8-wk EtOH mice had more inflammation, edema, and consolidation at days 5, 6, and 8 p.i. than that observed in water controls (Fig. 6 and data not shown; alveolar edema, \( p < 0.01 \); atelectasis, \( p < 0.01 \)). By day 8 p.i., while recovery was exhibited in the lungs of water mice (28.8% percent of the lung affected in day 8 groups vs 41.5% at day 6 groups by gross examination; Fig. 6A and data not shown), disease in EtOH mice continued to progress (53.5% of the lung affected in day 8 groups vs 46.5% in day 6 groups; Fig. 6B and data not shown). At the microscopic level, this difference in the severity of the lesion on day 8 p.i. was prominently localized to the alveoli with larger differences observed in alveolar atelectasis (water 1.8 ± 0.3, EtOH 3.2 ± 0.7; \( p < 0.05 \)) and edema (water 0.5 ± 0.3, EtOH 2.4 ± 0.7; \( p < 0.05 \)). Similar increases in histopathology scores were observed in the lungs of 4-wk EtOH infected mice (data not shown). Recently the increases in severe influenza disease have been observed to associate with an increase in neutrophilic infiltrate (25). Therefore, we enumerated the number of neutrophils present in the influenza lesions of 8-wk water and EtOH mice. Morphologically, as shown in Fig. 7, A and B and quantified in Fig. 7C, chronic EtOH consumption leads to increased neutrophil recruitment into the lungs during influenza virus infections.

Discussion

Our results demonstrate that chronic EtOH consumption reduces the number of, and by 8-wk of EtOH consumption, the effector ability of influenza-specific CD8 T cells. Although the suppression of CD8 T cells numbers during chronic EtOH has been described...
elsewhere (17, 20, 35), herein we show for the first time that extended chronic EtOH consumption (i.e., 8 wk+) further blocks the ability of virus-specific CD8 T cells to produce the effector molecule IFN-γ (Fig. 4, D and G). A similar dysregulation of pulmonary CD8 T cell responses has been described during respiratory syncytial virus (RSV) infections of naive (i.e., water-consuming) mice (38, 39). This loss in T cell effector function during RSV infections can be overcome by supplementation with IL-2 (39). Importantly, studies have shown reduced IL-2 production, rescue of defective T cell responses by exogenous IL-2 addition, and reduced T cell binding of IL-2 during acute alcohol consumption (40–42). To date, however, we have found no difference in pulmonary IL-2 levels or in CD25, CD122, or CD132 expression on pulmonary influenza-tetramer+ CD8 cells between the water and chronic EtOH groups (data not shown). Instead this dysregulation may relate to differential APC populations in the lungs of influenza-infected water and EtOH-exposed mice. In other systems, peripheral dendritic cell (DC) populations have been shown to influence Ag-specific T cell responses/dysregulation (43). Further, chronic EtOH is known to alter DC populations in the skin, spleen, and liver (44–49). Our preliminary results suggest that chronic EtOH likewise alters the DC cell numbers and subsets available in the lungs (data not shown). However, what role these changes may play, if any, in the altered immune response and disease severity found during influenza infection of chronic EtOH consuming mice described above awaits subsequent study.

Although the cellular and cytokine dependence of the EtOH suppression of IFN-γ CD8 T cell effector function remains unknown, we do know that the dysregulation is influenza-peptide specific, as PMA and ionomycin stimulation of these cells drives an equivalent % of CD8 T cells to produce IFN-γ (Fig. 5). This result suggests that the 8-wk EtOH-mediated defect is upstream of Ca2+ flux and PKC activation. Because we did not observe any differences in the intensity of tetramer staining on influenza-specific CD8 T cells (data not shown) from water and EtOH mice, it suggests that altered levels of cell surface TCR expression or reduced TCR affinity for influenza virus peptide-MHC complexes do not explain the inability of 8-wk EtOH CD8 T cells to produce IFN-γ. Recent studies have shown that EtOH reduces TLR signaling by altering recruitment and distribution of molecules in lipid rafts (50). Because recruitment and redistribution of molecules into lipid rafts is likewise an important facet of TCR-mediated signaling (51, 52), these results suggest that the defect leading to EtOH-mediated IFN-γ dysregulation in T cells may ultimately lie in lipid rafts and signaling molecules downstream of TCR engagement but upstream of PKC and Ca2+ flux.

Given the peptide-MHC specific nature of TCR-dependent IFN-γ production by CD8 T cells, the EtOH-induced IFN-γ defect would only be observed when directly examining Ag-specific effector responses. Whether this EtOH-induced dysregulation is unique to influenza-specific T cells or is more global and occurs with pulmonary T cells or CD8 T cell responses in general remains in question. Interestingly, a recent study using the same EtOH in drinking water model has shown a similar loss of IFN-γ production by listeriolysin O-specific CD8 T cells in the spleen following Listeria monocytogenes infections (P. Gurung, B. M. Young, R. A. Coleman, S. Wiechert, L. E. Turner, N. B. Ray, T. J. Waldschmidt, K. L. Legge, and R. T. Cook, manuscript in preparation). Together these results suggest that chronic EtOH may alter the effector abilities of CD8 T cells in many tissues possibly via alteration of TCR signaling or in APC subset availability.

The mechanism regulating the overall global decrease in T cells in chronic EtOH mice remains unknown at this time, but previous studies have demonstrated that T cells in alcoholics and animal models show increased levels of activation in the unimmunized state (17, 20, 53). In turn, this chronic activation of the T cell pool may alter the T cell’s ability to expand and respond to subsequent viral challenges (i.e., anergy), place the T cells under increased regulatory cell control, or lead to their elimination through activation-induced cell death. Such mechanisms would decrease the overall numbers of T cells available to respond to the influenza virus challenge. Consistent with this idea, we did observe reduced numbers of both total CD8 T cells and influenza-specific CD8 T cells in the lung draining lymph nodes on day 8 p.i. (data not shown). This suggests that the reduced pulmonary response observed herein could relate to altered precursor frequency, activation, or expansion of influenza-specific CD8 T cells within the lymph nodes. Our observation of decreased numbers of total CD8 T cells in the lungs following influenza virus infections is consistent with the results of Shellito and Olariu (35), who observed decreased numbers of CD8 T cells in the lungs of EtOH mice following Pneumocystis carinii infections. This loss of CD8 T cells from the lungs was not mirrored by a similar loss of these cells in the spleen, and therefore, the differential CD8 T cell numbers were ascribed to be related to altered migration of the T cells into EtOH lungs (35). In contrast, we found that the decreased numbers of CD8 T cells in the lungs of influenza virus-infected EtOH mice were paralleled by similar losses of these cells in the spleens and lymph nodes (data not shown), suggesting that, in our case, altered migration of the T cells from the lymphoid organs into the lungs may not explain the observed differences. At this time, the relative contributions of these potential mechanisms to the observed defect in CD8 T cell recruitment and influenza virus-specific CD8 T cell immunity specifically in chronic EtOH vs water environments awaits further study.

All together, the results presented herein suggest that chronic EtOH exposure reduces adaptive immunity and thereby increases pulmonary viral titers, inflammation, and disease severity. Further, given the reduced levels of influenza-specific CD8 T cells and the known alterations that EtOH exposure has on pulmonary resident cells like alveolar macrophages (8, 54), it is likely that the overall inflammatory environment in the lungs of water and EtOH mice are distinct and contribute to the observed pathology. In this regard, highly lethal influenza infections are thought to be associated with high levels of inflammatory cytokines (37, 55). Moreover, recent studies have suggested that the increased neutrophilic infiltration associated with experimental 1918 influenza A infections may, in part, be responsible for the increased inflammatory cytokine and chemokine expression (25). Consistent with those studies, we have also observed increased neutrophilia and in a preliminary experiment marked increases in inflammatory cytokine mRNA, including type I IFNs and IL-17F, in the lungs of influenza-infected EtOH mice (data not shown). Therefore, collectively, the 1918 influenza virus studies together with our own data presented herein suggest that the increased pathology associated with influenza infection of EtOH mice could in part relate to the increased pulmonary neutrophilia. However, the benefits of blocking or reducing this neutrophilia in EtOH mice may be limited as removal of neutrophils from 1918 infected mice led to higher viral titers and similar lethality (25). Therefore, the increased pulmonary neutrophilia observed herein and in other lethal influenza virus infections (25) may instead be indicative of a compensatory innate immune response geared at limiting viral infection during times of reduced influenza-specific CD8 T cell immunity.

In addition to the increased neutrophilia, our results show that the pulmonary lesions of influenza-infected EtOH consuming mice were routinely associated with marked edema (Fig. 6G). The delicate fluid balance in the airways and alveolar epithelia is regulated.
largely by active electrolyte transport (56). Epithelial tight junctions additionally regulate paracellular permeability. Previous studies have demonstrated that chronic EtOH consumption results in loss of epithelial tight junction proteins and increased paracellular permeability. This leakage is related to a cascade of factors including the loss of glutathione, concomitant increases in oxidative stress, up-regulation of TGF-β, and reduced GM-CSF receptor signaling (56). Furthermore, influenza virus infection alone is known to inhibit amiloride-sensitive sodium channel activity and increase fluid retention in the lungs (57). Therefore, the marked edema observed within the lungs of influenza-infected EtOH consuming mice may reflect both alcohol-induced increased epithelial permeability as well as viral-induced fluid retention.

Our observed increases in pulmonary viral loads, inflammation, edema, and neutrophilia as well as decreased numbers of dendritic cells in the lungs of influenza infected EtOH mice are consistent with the recent findings of Jerrells et al. (58) during pulmonary infections of EtOH mice with RSV. However, unlike the influenza infection data presented herein, the neutrophilia in that work was more predominating during the first 24- to 48-h p.i. and dissipated at later times (i.e., day 5 post-RSV infection). Further, while we have observed a decrease in total lymphocytes in the lungs of influenza-infected animals, Jerrells et al. (58) observed an increased number of pulmonary lymphocytes in EtOH mice on day 5 post RSV infection. It should be noted that the RSV studies described above were focused upon the bronchial alveolar lavage fluid rather than the full lung environment (i.e., bronchial alveolar lavage fluid and lung interstitium) examined herein. This difference in focus may in part explain the differences in kinetics and lymphocyte numbers between RSV and influenza virus infections of EtOH-conditioned mice. Alternatively, these differences could instead relate to potency of the various virus infections in mice, as infectious RSV virions were cleared from the lungs during the first 48–72 h of the infection (58), while influenza virus infections persist for 5–8 days in water control mice (Fig. 2) (24). Regardless, the similarities between the two models suggest that pulmonary infections of alcohols with a seasonal respiratory virus, such as RSV and influenza, would lead to increased disease severity that featured increased neutrophilia, inflammation, and edema as well as sustained viral titers and reduced pulmonary APC numbers.

In closing, we have shown that chronic EtOH consumption increases the risk for severe disease and death during influenza infections. This increased severity appears multifactorial and includes increased edema and neutrophilia and a loss of competent influenza-specific effector T cells. Given the distinct role CD8 T cells in limiting virus replication and mortality in mice, strategies designed to boost CD8 T cell immunity to this important human pathogen in these individuals could reduce both the burden and severity of influenza infections and lead to more desirable outcomes.

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