achieve further improvements in RT-qPCR, whereas switching to different qPCR master mix solutions has a negative effect on Ct values (Table E1).

Finally, we compared the original and improved protocols with regard to both RNA isolation and RT-qPCR. A 7.4-fold increase in RNA yield was achieved by using the optimized RNA isolation protocol (Figure 1E). Moreover, optimization of the qPCR protocol resulted in significantly lower Ct values of GAPDH amplicons of all sizes compared with the original protocol (Table E2).

In conclusion, we have developed a combined sputum processing/RNA extraction/RT-qPCR protocol to maximize the quantity and quality of RNA obtained from sputum, and to enhance detection of transcripts by qPCR. Successful elimination of bacterial DNA was shown to be a critical step in the optimization process. Our results should facilitate future gene-expression studies utilizing sputum. ■

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Preexisting Type 2 Immune Activation Protects against the Development of Sepsis

To the Editor:

Sepsis is a clinical syndrome of life-threatening organ dysfunction caused by a dysregulated host immune response to infection (1). Given the importance of immune responses in acute sepsis pathophysiology, we speculated that the immune responses associated with preexisting comorbidities would impact the development of sepsis during acute infection.

Using the Truven MarketScan private insurance claims database of more than 150 million patients, we identified 73,587 patients with sepsis based on the *International Classification of Diseases, Ninth Revision, Clinical Modification* (ICD-9-CM) codes for sepsis (995.91) or severe sepsis (995.92). We matched 5:1 nonseptic patients to septic patients by age, sex, location, and number of comorbid diagnoses. We then determined the prevalence of various immune-mediated diseases based on ICD-9-CM codes using a previously published methodology (2). Although the choice of immune-mediated diseases was arbitrary, a number of immunologic diseases were specifically excluded from the results because they either were congenital (and therefore present throughout the life of the patient) or have a poorly understood pathophysiology. Patients were considered to have a comorbid immunologic disease if they had ever had such a diagnosis during the time period captured in the database, in either inpatient or outpatient encounters. Once the prevalence of these comorbid immune conditions was determined, the odds ratios were calculated between septic and nonseptic groups and compared using Fisher's exact test. P values for significant associations were adjusted for multiple test comparison (MTC) using the Benjamini-Hochberg correction with a false discovery rate threshold of 0.1%, with a resultant P value MTC < 0.05 considered significant (3). In

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Table 1, the data are expressed both as absolute numbers and as percentages of the total in parentheses. Odds ratios are expressed with their confidence intervals, determined before MTC.

Interestingly, a number of immune-mediated diseases, including vasculitis, ulcerative colitis, multiple sclerosis, type 1 diabetes, and (among females) lupus erythematosus, were *overrepresented* among septic patients, suggesting that these diseases predispose patients to the dysregulated immune response of sepsis. However, to our surprise, diseases associated with an overactive type 2/T-helper cell type 2 (Th2) immune response, such as asthma, allergic rhinitis, atopic dermatitis, and food allergy, were markedly and significantly *underrepresented* among septic patients. This discovery suggested to us that these diseases protect patients from becoming septic.

To determine the biological plausibility of this hypothesis, we induced pulmonary type 2 inflammation in two different mouse models of allergic asthma followed by systemic infection with *Staphylococcus aureus* to cause lethal sepsis. This model recapitulates many of the histopathologic features observed in patients with *S. aureus* sepsis, including lung injury and liver pathologic changes (4). Innate type 2 immune responses were induced by 3 days of intratracheal IL-33 (100 ng) administration to C57BL/6 mice, which causes airway hyperreactivity, eosinophilia, and goblet cell hyperplasia independently of the adaptive immune system (5). On Day 4, the mice were infected with 5×10^7 CFU of *S. aureus* USA300 LAC/100 μl via retro-orbital injection (4). Separately, adaptive T cell–dependent type 2 airway inflammation was induced by 100 μg intratracheal house dust mite (HDM) sensitization (Day 0) and challenge (Days 7–10 with 25 μg HDM/day), followed by retro-orbital injection with *S. aureus* on Day 14 (6). In both models, the presence of type 2 airway inflammation before infection was confirmed by flow cytometry (data not shown). Although 85% of control mice were dead by Day 5, pretreatment with IL-33 to induce innate type 2 inflammation significantly protected the mice from mortality, with 50% of the mice still alive by Day 7 (Figure 1). More dramatically, HDM sensitization and challenge to induce adaptive Th2 responses before infection robustly protected against *S. aureus*–induced mortality, with 75% of the mice still alive by Day 7. Thus, the presence of a preexisting type 2–biased immune response protected the mice from becoming septic and dying, confirming our hypothesis generated from the insurance claims analysis.

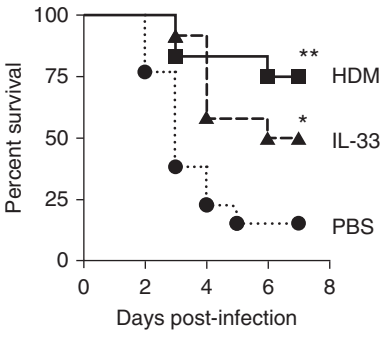

Figure 1. Preexisting type 2 immune responses protect against *Staphylococcus aureus*–mediated mortality in mice. C57BL/6 mice (6–7 weeks old, 15–17 g) were administered intratracheal IL-33 or were sensitized and challenged with house dust mite (HDM) before they were intravenously infected with a lethal dose of *S. aureus* USA300. Controls received PBS. Shown are two pooled experiments for each group, with a total of 12–13 mice per group. Mice were killed when they reached 70% starting weight according to protocol or on Day 7. Survival curves were analyzed using the log-rank test (GraphPad Prism 6). Significance was determined at $P < 0.05$. PBS versus IL-33, $^*P = 0.01$; PBS versus HDM, $^{**}P = 0.001$.

Our "big data" analysis allowed us to infer a novel disease mechanism with significant implications for our understanding of sepsis pathophysiology. Our confirmation of this mechanism using two different mouse models establishes a new paradigm for translational sepsis research in which analysis of patterns in administrative data generates hypotheses to be tested using reductionist mouse approaches. These findings demonstrate that an individual's immune status before infection has a significant impact on her/his risk for the development of sepsis, and may also impact the disease course and outcomes.

During sepsis, activation of type 1 or type 17 inflammatory responses leads to the robust production of proinflammatory cytokines such as IL-6, TNF-α, IFN-γ, and IL-17A. The "cytokine storm" of this response mediates cardinal features of septic inflammation, including phagocyte recruitment to the site of infection, pathogen elimination, organ dysfunction, and inadvertent host tissue destruction (7). In addition, these type 1/type 17 pathways are aberrantly activated in the immune diseases that confer an increased risk of developing sepsis, supporting the importance of preexisting immune diseases for sepsis risk (8).

The surprising finding that allergic diseases were underrepresented in our septic patients suggests a protective effect on sepsis development and/or outcomes. Overactive type 2 immune responses can antagonize proinflammatory type 1 and 17 responses, and are often considered anti-inflammatory and tissue reparative (7). For instance, both type 2 innate lymphoid cells and Th2 lymphocytes in the lung produce amphiregulin, an epithelial growth factor that restores tissue integrity after injury associated with inflammation (9). Understanding the role of innate and adaptive type 2 responses before and during septic inflammation will inform the development of improved risk-prediction algorithms and reveal novel therapeutic targets.

We did not adjust for medication use in our analysis; however, nearly all of the diseases listed in Table 1 are treated with local or systemic immunosuppression, which may reduce the significance of medications as a confounder. Our mouse models were not subjected to immunosuppressive or antimicrobial medications, and they did not have the genetic and comorbid-illness heterogeneity observed in humans. Nevertheless, the clustering of *only* type 2 diseases as protective in the claims dataset and the robust protection noted in the mouse models suggests mechanistic specificity.

In summary, we combined analysis of large-scale administrative data with analysis of mouse models to reveal a novel, unappreciated immunologic disease mechanism in sepsis. Consideration of baseline immunologic bias as manifested by comorbid illnesses may predict hospital courses and outcomes for acutely infected patients, and modulating type 2 responses may improve those outcomes.

Some of the results of these studies have been previously reported in the form of an abstract (10). ■

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Table 1. Odds of Immune-Mediated Diseases among Septic and Nonseptic Patients

	Septic	Nonseptic	Odds Ratio (CI)	*P* Value (MTC)
Males, total *n* (%)	36,594 (100)	182,970 (100)		
Sjogren's syndrome	42 (0.11)	460 (0.25)	0.456 (0.410–0.557)	1.7E-4
*Food allergy	187 (0.5)	1,942 (1.1)	0.479 (0.410–0.557)	1.48E-22
*Allergic rhinitis	4,046 (11.1)	37,244 (20.4)	0.486 (0.470–0.528)	5.18E-296
Graves disease	131 (0.4)	976 (0.5)	0.670 (0.553–0.804)	0.016
*Asthma	4,027 (11.0)	28,027 (15.3)	0.684 (0.660–0.708)	2.73E-103
*Atopic and contact dermatitis	5,316 (14.5)	34,115 (18.7)	0.742 (0.719–0.765)	6.86E-78
Celiac disease	104 (0.3)	694 (0.4)	0.749 (0.603–0.921)	ns
Sarcoidosis	253 (0.7)	1,408 (0.8)	0.898 (0.781–1.028)	ns
Dermatomyositis and polymyositis	114 (0.3)	585 (0.3)	0.974 (0.790–1.193)	ns
Lupus erythematosus	296 (0.8)	1,457 (0.8)	1.016 (0.893–1.152)	ns
Myasthenia gravis	84 (0.2)	410 (0.2)	1.024 (0.800–1.300)	ns
Type 1 diabetes mellitus	5,604 (15.3)	22,490 (12.3)	1.290 (1.250–1.332)	2.22E-50
Vasculitis	597 (1.6)	2,222 (1.2)	1.349 (1.230–1.478)	8.51E-7
Ulcerative colitis	934 (2.6)	3,317 (1.8)	1.419 (1.317–1.527)	4.63E-16
Multiple sclerosis, other demyelinating diseases	601 (1.6)	1,808 (1.0)	1.673 (1.522–1.837)	1.12E-21
Kawasaki disease	30 (0.08)	54 (0.03)	2.780 (1.717–4.422)	0.044
Females, total *n* (%)	36,993 (100)	184,965 (100)		
*Allergic rhinitis	5,698 (15.4)	54,005 (29.2)	0.442 (0.429–0.455)	7.99E-269
*Food allergy	281 (0.8)	2,741 (1.5)	0.509 (0.448–0.576)	3.01E-28
Sjogren's syndrome	363 (1.0)	2,958 (1.6)	0.610 (0.545–0.681)	1.44E-17
Graves disease	357 (1.0)	2723 (1.5)	0.652 (0.582–0.729)	4.70E-12
Celiac disease	186 (0.5)	1,378 (0.8)	0.673 (0.574–0.786)	2.75E-4
*Atopic and contact dermatitis	6,729 (18.2)	45,297 (24.5)	0.686 (0.666–0.705)	1.73E-153
*Asthma	6,484 (17.5)	42,386 (22.9)	0.715 (0.694–0.736)	1.29E-116
Sarcoidosis	429 (1.2)	2,155 (1.2)	0.995 (0.895–1.105)	ns
Myasthenia gravis	116 (0.3)	555 (0.3)	1.045 (0.848–1.279)	ns
Systemic lupus erythematosus	1,515 (4.1)	6,286 (3.4)	1.214 (1.146–1.286)	1.47E-7
Dermatomyositis and polymyositis	214 (0.6)	839 (0.5)	1.277 (1.093–1.486)	ns
Vasculitis	926 (2.5)	3230 (1.7)	1.445 (1.340–1.556)	9.35E-18
Multiple sclerosis, other demyelinating diseases	1,163 (3.1)	3,959 (2.1)	1.484 (1.388–1.586)	5.57E-26
Ulcerative colitis	1,140 (3.1)	3,802 (2.1)	1.515 (1.415–1.621)	3.74E-28
Type 1 diabetes mellitus	5,184 (14.0)	15,927 (8.6)	1.730 (1.672–1.789)	8.87E-205
Kawasaki disease	25 (0.07)	31 (0.02)	4.034 (2.283–7.061)	1.61E-3

Definition of abbreviations: CI, confidence interval; MTC, multiple test comparison; ns, not significant.
*Diseases considered type 2–mediated.
CI refers to the confidence intervals calculated by Fisher's exact test before MTC correction. *P* value (MTC) refers to the MTC-corrected *P* value, with significance at $P < 0.05$. The MTC values for nonsignificant comparisons may be greater than one and therefore are not included in this table.

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