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Report

An Endonuclease Allows *Streptococcus pneumoniae* to Escape from Neutrophil Extracellular Traps

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Summary

Streptococcus pneumoniae **(pneumococcus) is the most common cause of community-acquired pneumonia, with high morbidity and mortality worldwide [1]. A major feature of pneumococcal pneumonia is an abundant neutrophil infiltration [2]. It was recently shown that activated neutrophils release *n*eutrophil *e*xtracellular *t*raps (NETs), which contain antimicrobial proteins bound to a DNA scaffold. NETs provide a high local concentration of antimicrobial components [3] and bind, disarm, and kill microbes extracellularly [4]. Here, we show that pneumococci are trapped but, unlike many other pathogens, not killed by NETs. NET trapping in the lungs, however, may allow the host to confine the infection, reducing the likelihood for the pathogen to spread into the bloodstream. DNases are expressed by many Gram-positive bacterial pathogens [5, 6], but their role in virulence is not clear. Expression of a surface endonuclease encoded by *endA* [7] is a common feature of many pneumococcal strains. We show that EndA allows pneumococci to degrade the DNA scaffold of NETs and escape. Furthermore, we demonstrate that escaping NETs promotes spreading of pneumococci from the upper airways to the lungs and from the lungs into the bloodstream during pneumonia.**

Results

Pneumococci Are Captured but Not Killed by NETs In Vitro

The pneumococcal strain TIGR4 (serotype 4) belongs to a clonal type with high capacity to cause invasive disease in humans [8]. TIGR4 pneumococci interact with neutrophil extracellular traps (NETs). Figures 1A–1B show the filamentous NET structures stained for DNA (blue) and the granular enzyme neutrophil elastase (NE) (red). Pneumococci (green) are captured by NETs (arrows) in a dose-dependant fashion (Figures 1A–1B).

We tested whether NETs kill pneumococci (Figure 1C). To distinguish between phagocytic and NET microbicidal activity, we blocked phagocytosis with the actin-polymerization inhibitor Cytochalasin D before infecting neutrophils [4, 9, 10] (see Figure S1 in the Supplemental Data available online). TIGR4 pneumococci were completely resistant to NET killing. The Gram-negative bacteria *Shigella flexneri* served as a positive control and were efficiently killed by NETs (Figure 1C).

Pneumococcal Endonuclease EndA Degrades Extracellular DNA

We demonstrated that TIGR4 pneumococci degrade extracellular DNA by incubating bacteria with salmon sperm DNA (Figure 2A). The DNase activity was almost exclusively associated with the bacteria, and only marginal activity could be detected in the culture supernatant (Figure S2). We identified the pneumococcal gene *endA* in the TIGR4 genome as a likely homolog of the *Streptococcus pyogenes* DNase genes *spd* and *sda* [5]. *endA* is known to encode a membrane bound nuclease (TIGR4 SP1964) important for DNA uptake [7, 11, 12]. We made the TIGR4 isogenic knockout, TIGR4Δ(*endA*), and showed that it failed to degrade extracellular DNA (Figure 2A), although the mutant grew as efficiently as wild-type TIGR4 in vitro (Figure S3). Reintroducing *endA* into TIGR4Δ(*endA*) generated TIGR4Δ(*endA*)∇(*endA*) and restored the DNase activity (Figure 2A). TIGR4Δ(*endA*), like the wild-type, was not killed by the antimicrobial activity of NETs (Figure 1C). Our data show that EndA represents a major nuclease that allows TIGR4 pneumococci to degrade extracellular DNA efficiently.

Pneumococci Destroy NETs

Because the scaffold of NETs is DNA [4], we tested whether pneumococcal EndA affects NET integrity. Activated neutrophils were incubated with either culture medium RPMI (Figure 2B), bovine pancreatic DNase (Figure 2C), TIGR4Δ(*endA*) (Figure 2D), or TIGR4 (Figure 2E). After 30 min incubation, the samples were fixed and stained for DNA (blue) and NE (red). NETs were intact in cells incubated with medium but disintegrated after incubation with DNase or TIGR4. In contrast, infection with TIGR4Δ(*endA*) did not affect the NET integrity.

We also examined the disintegration of NETs by EndA with a functional assay using *S. flexneri* as a sensitive reporter for the antimicrobial activity of NETs (Figure 2F). We first exposed NETs to culture medium, bovine pancreatic DNase, TIGR4, or TIGR4Δ(*endA*) and then infected them with *S. flexneri*, which, unlike pneumococci, are sensitive to NET killing (Figure 1C). Figure 2F shows that NETs exposed to culture medium (control) killed around 50% of the *S. flexneri* inoculum. Bacterial killing

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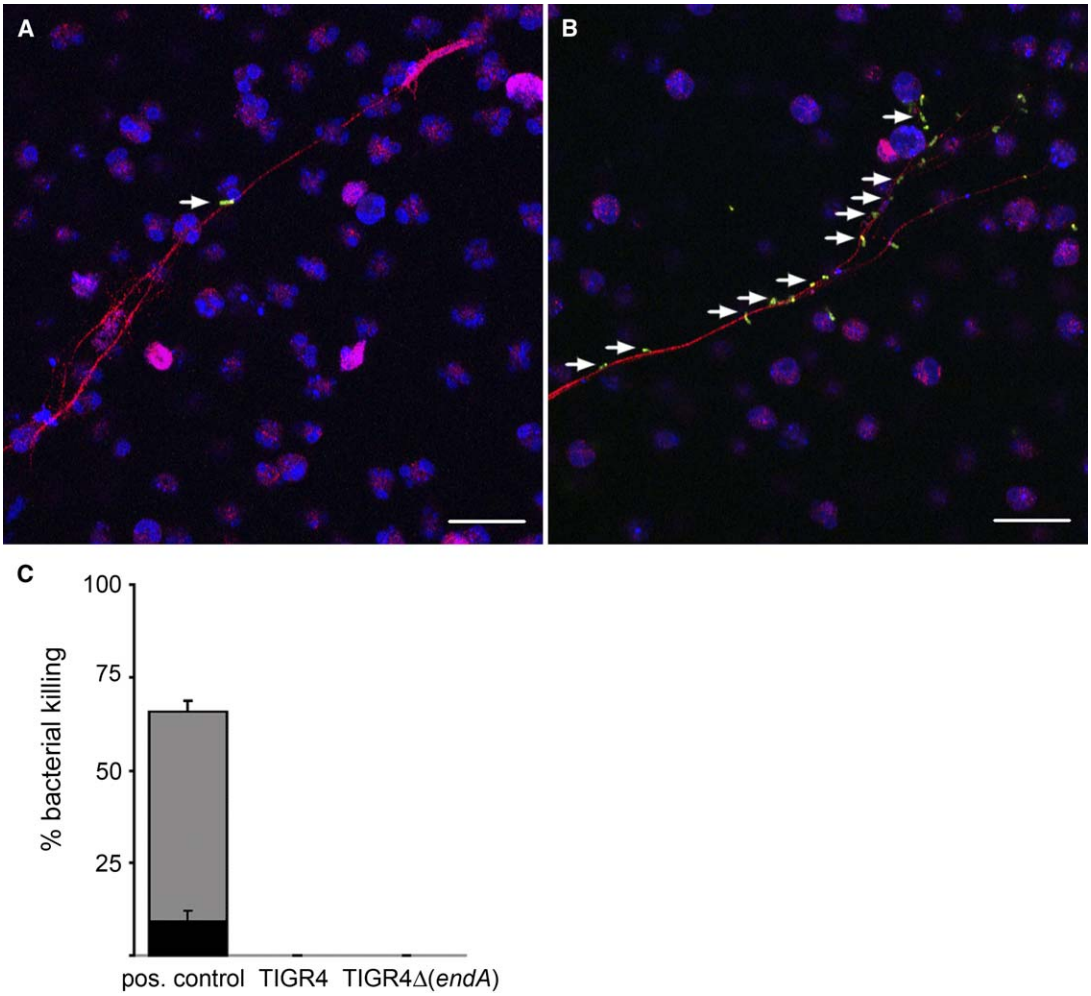

Figure 1. *S. pneumoniae* Is Trapped but Not Killed by NETs In Vitro

(A and B) Neutrophils were stimulated with PMA and infected with FITC-labeled TIGR4 pneumococci (green) at a multiplicity of infection (MOI) of 1 (A) or 100 (B). Five minutes postinfection, the samples were fixed and stained for DNA (blue) and neutrophil elastase (NE, red). Scale bars represent 20 μm. NETs are identified as filamentous structures. The dose-dependent trapping of pneumococci (arrows) in NET structures (MOI 1 versus MOI 100) can be observed.

(C) The percentage of bacterial killing by NETs (gray) and by phagocytosis (black), which together make up total killing, is shown for *Shigella flexneri* (pos. control) and pneumococci [TIGR4, TIGR4Δ(*endA*)]. Mean and standard error of the mean (SEM) are shown both for total and for phagocytosis killing. Assays were performed at least three independent times for each strain. Pneumococci were killed neither by NETs nor by phagocytosis.

was reduced to around 30% after treatment with bovine DNase. NETs exposed to TIGR4 also only killed around 30% of *S. flexneri* inoculum. Significantly, NETs exposed to TIGR4Δ(*endA*) killed as efficiently as the control.

In a further demonstration that EndA degrades NETs, the release of NE was used as an indicator. This enzyme is normally bound to NETs and found at very low concentrations in culture supernatants. Indeed, a very low NE level was detected in the supernatant of NETs incubated with culture medium (Figure 2G, control). The concentration of NE increased in the supernatant when NETs were incubated with DNase or TIGR4. Significantly, very low NE concentrations were found in the supernatant of NETs incubated with TIGR4Δ(*endA*). The released NE came exclusively from NETs and not from intact neutrophils because DNase treatment did not increase NE in the supernatant of unstimulated neutrophils (Figure S4). Also, in the absence of DNase, stimulated neutrophils showed low NE levels in the supernatant. Taken together, these three approaches demonstrate that EndA degrades the DNA scaffold of NETs and thereby destroys their functional integrity.

EndA Allows Pneumococcal Escape from NETs

To analyze the effect of DNase activity on pneumococcal trapping, we infected activated neutrophils with TIGR4, TIGR4Δ(*endA*), and TIGR4Δ(*endA*)∇(*endA*). Five (Figures 3A–3C) and thirty (Figures 3D–3F) minutes after infection, samples were fixed and stained for pneumococci (green), DNA (blue), and NE (red). Five minutes postinfection (p.i.), similar numbers of bacteria of all three strains were associated with NETs (arrows). Thirty minutes p.i., however, there were very few NETs in cultures infected with TIGR4 or TIGR4Δ(*endA*)∇(*endA*), and the bacteria were lost in the wash. In contrast,

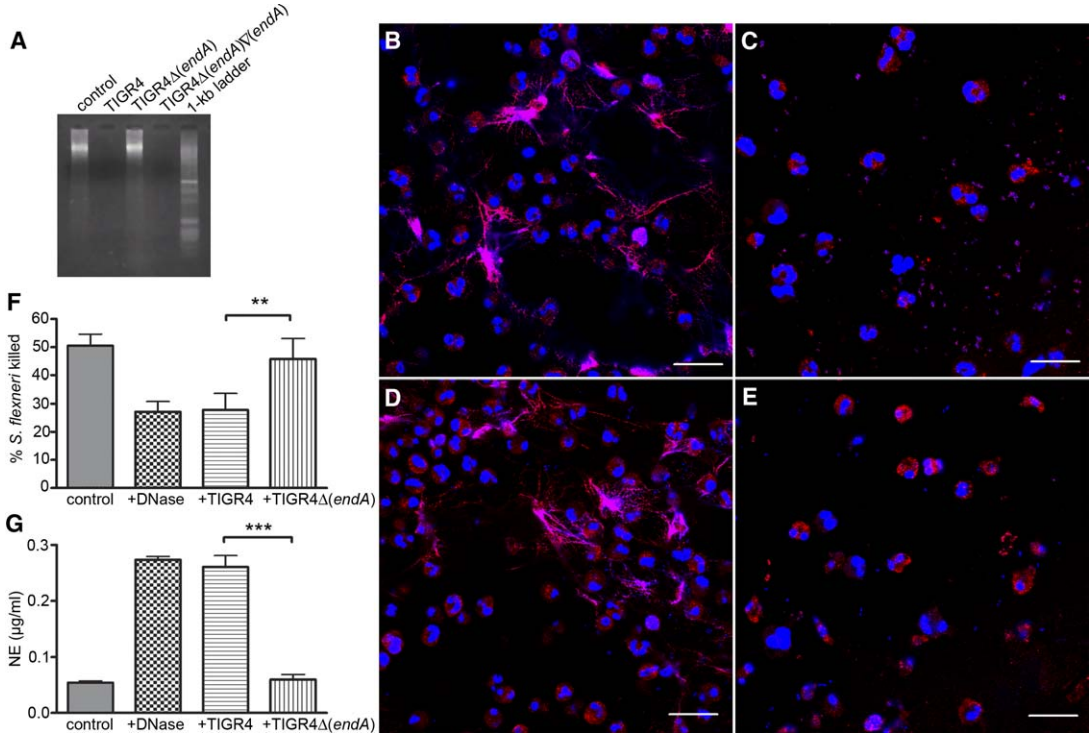

Figure 2. EndA Is a DNase that Degrades Neutrophil Extracellular Traps

(A) Salmon sperm DNA was incubated with TIGR4, TIGR4Δ(*endA*), and TIGR4Δ(*endA*)∇(*endA*) and resolved on an agarose gel. A sample without pneumococci was used as negative control. DNA incubated with TIGR4 or TIGR4Δ(*endA*)∇(*endA*) was degraded. No degradation was observed when DNA was incubated with TIGR4Δ(*endA*), which lacks the DNase gene.

(B–E) Neutrophils were activated to make NETs and treated with medium (control) (B), bovine pancreatic DNase (C), TIGR4Δ(*endA*) pneumococci (D), or TIGR4 pneumococci (E). The samples were stained for DNA (blue) and NE (red). Scale bars represent 20 μm. NETs are degraded in samples treated with bovine DNase or TIGR4 pneumococci.

(F) NET-mediated killing of the reporter strain of *S. flexneri*. The NETs were exposed to RPMI medium (control), bovine pancreatic DNase, TIGR4, or TIGR4Δ(*endA*) pneumococci. We measured killing of *S. flexneri* as a reporter of NET antimicrobial activity. Mean and SEM are shown. The assay was performed three times and analyzed with the nonparametric Mann-Whitney test. A p value < 0.05 was considered significant. *Shigella flexneri* were killed less efficiently after NETs were treated with DNase or DNase-producing pneumococci.

(G) NETs were treated with RPMI medium (control), bovine pancreatic DNase, TIGR4, or TIGR4Δ(*endA*) pneumococci. We measured the NE concentration in the supernatant as a reporter of NET degradation. Mean and SEM are shown. The assay was performed three times and analyzed with the nonparametric Mann-Whitney test. A p value < 0.05 was considered significant. Higher concentrations of NE could be measured in the supernatant of neutrophils cultures after exposure to TIGR4 compared to TIGR4Δ(*endA*), indicating that the TIGR4-encoded DNase EndA degraded NETs, thereby releasing NE.

abundant TIGR4Δ(*endA*) bacteria remained associated with intact NETs (Figure 3E).

NETs Are Made in Pneumococcal Pneumonia

To study NET formation in vivo, we infected C57BL/6 mice intranasally with the TIGR4 strain to induce pneumonia. The animals were sacrificed 48 hr p.i., and lungs were stained for the NET markers DNA (blue), histone H1 (green), and a 40 kDa neutrophil-specific surface marker (red). In mock-infected mice (Figures 4A and 4C), DNA and histone H1 both were restricted to nuclei. In contrast, in TIGR4-infected lungs, an influx of activated neutrophils, accompanied by extracellular DNA and histone H1, was observed (Figures 4B and 4D). The NETs localized to alveoli, as shown in the merged image. Similar results were obtained after infection with TIGR4Δ(*endA*) (data not shown). A mean of 48% (± 18% standard deviation [SD]) of the inspected lung fields contained NETs after infection with TIGR4 as quantified by analyzing a total of 364 fields (40× objective) in the lungs of ten different mice. No NETs were observed in mock-infected animals.

NET Destruction Affects *S. pneumoniae* Virulence

The role of EndA in vivo was tested by comparing infections with TIGR4, TIGR4Δ(*endA*), and TIGR4Δ(*endA*)∇(*endA*) after intranasal inoculation (Figure 4E). The *endA* mutant exhibited a delayed onset of severe disease, and at 65 hr postinfection, mice infected with the *endA* mutant had around 60% survival whereas animals infected with either TIGR4 or its isogenic revertant TIGR4Δ(*endA*)∇(*endA*) had around 40% survival. These differences had a p value lower than 0.05 by a Kaplan-Meier-analysis log-rank test.

We also investigated the role of *endA* in virulence by infecting mice with two strains simultaneously and determining the competitive index (CI). The CI is the ratio of the number of bacteria recovered from each strain in the same organ (e.g., lungs) and determines the relative fitness of an individual strain to survive in that organ [13]. A CI of 1 is obtained when both strains are recovered in equal numbers from a specific organ and indicates that both strains are equally fit. A CI of 0.1 is obtained when 10-fold less bacteria are recovered from one strain than from the other, and it indicates that the strain is

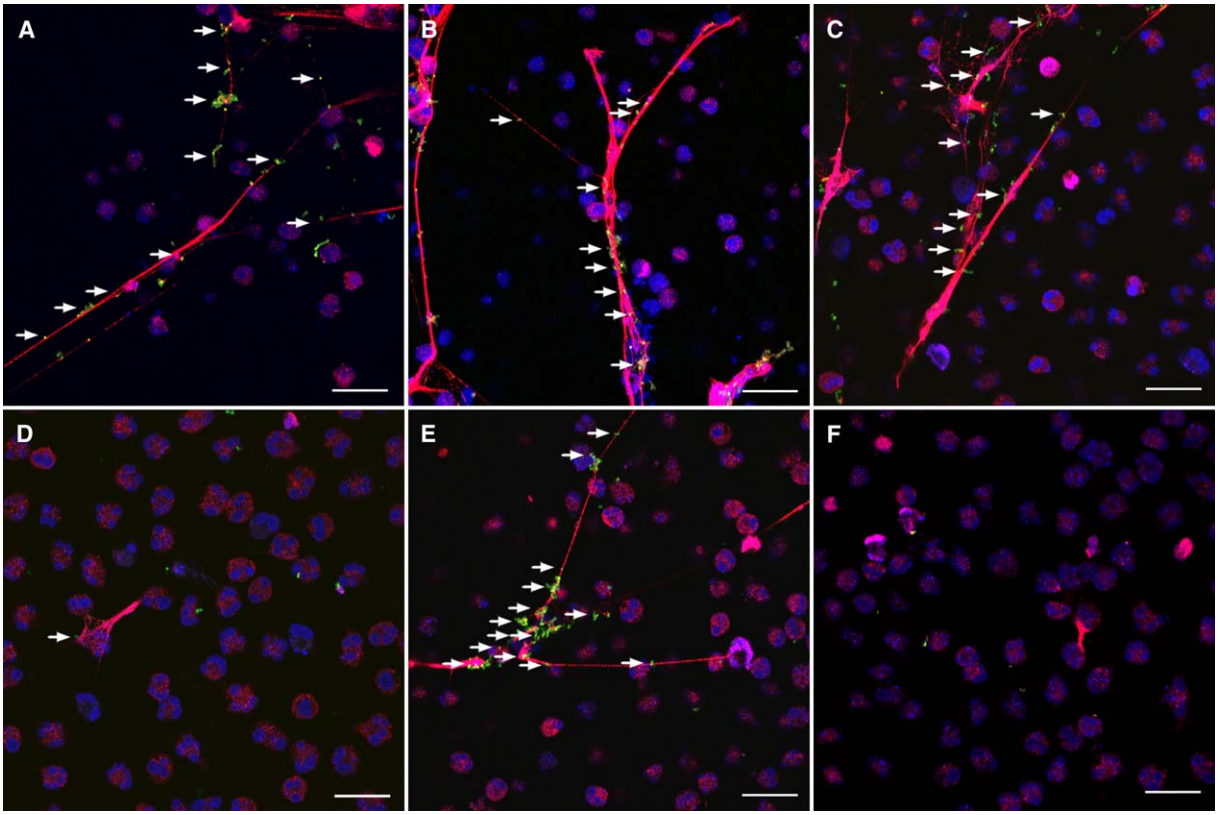

Figure 3. EndA Allows Pneumococci to Escape NETs

Neutrophils were activated to make NETs and infected at a MOI of 100 with TIGR4, TIGR4Δ(*endA*), or TIGR4Δ(*endA*)∇(*endA*). Samples were fixed 5 (top panels) or 30 min (bottom panels) after infection and stained for DNA (blue) and NE (red). Pneumococci were labeled with FITC (green) before infection. Scale bars represent 20 μm. Five minutes p.i., NET formation was observed in all samples (A–C), and similar numbers of TIGR4 (A), TIGR4Δ(*endA*) (B), and TIGR4Δ(*endA*)∇(*endA*) (C) were initially associated with NETs (arrows). Thirty minutes after infection, only TIGR4Δ(*endA*) pneumococci were still bound to NETs (E), whereas the EndA-expressing TIGR4 (D) and TIGR4Δ(*endA*)∇(*endA*) (F) detached from the strongly degraded NETs and were washed away. Thus, *endA*-encoded DNase activity liberates bacteria from NETs.

severely attenuated. Furthermore, by comparing the CI from different organs, it is possible to analyze how successfully a strain spreads within the host. This approach is possible because EndA is not secreted by pneumococci and therefore cannot rescue the *endA* mutant [7, 12] (Figure S2).

Mice were infected intranasally with TIGR4 and TIGR4Δ(*endA*) in a 1:1 ratio, and the bacterial load was determined in the upper respiratory tract (URT), the lungs, and the bloodstream. Figure 4F shows that at a terminal stage of pneumonia, similar numbers of TIGR4 and TIGR4Δ(*endA*) were recovered from the URT (CI ~1), indicating that both strains are equally competent in colonizing the upper airways. In contrast, 9–12 times more TIGR4 than TIGR4Δ(*endA*) bacteria were recovered from the lungs and from the bloodstream (CI = 0.11 and 0.08, respectively). Already 24 hr after infection, there were four times more TIGR4 than mutant bacteria in the bloodstream (CI = 0.23, Figure 4G). This difference was even more pronounced at 48 hr p.i., with 12.5 times as many TIGR4 (CI = 0.08, Figure 4G). A competition experiment between TIGR4Δ(*endA*) and TIGR4Δ(*endA*)∇(*endA*) yielded similar results (Figures 4H–4I), demonstrating that the phenotype of TIGR4Δ(*endA*) is specific for the *endA* mutation. These data strongly suggest that although TIGR4Δ(*endA*) is still able to colonize the upper airways to the same extent as TIGR4, it is less efficient in reaching and propagating in the lungs and in the bloodstream.

Many Different *S. pneumoniae* Strains Degrade NETs

S. pneumoniae comprises many different serotypes and clonal types. To determine whether degradation of NETs is a common virulence attribute, we tested pneumococcal strains of seven different capsular types (serotypes 1, 2, 4, 7F, 9V, 14, and 19F). This collection represents strains belonging to clonal types with different capacity to cause invasive disease in humans [8, 14]. All the strains tested, except one belonging to a clone of serotype 1, showed strong EndA activity as tested on salmon sperm DNA (Figure 5A). Analogously, all strains with EndA activity degraded NETs, as measured by the release of NE (Figure 5B). This shows that NET degradation is a common feature of pathogenic pneumococci.

Discussion

Streptococcus pneumoniae is the main cause of community-acquired pneumonia, and in 20%–30% of these cases, bacteria spread to the bloodstream [15]. The main clinical characteristic of pneumococcal pneumonia

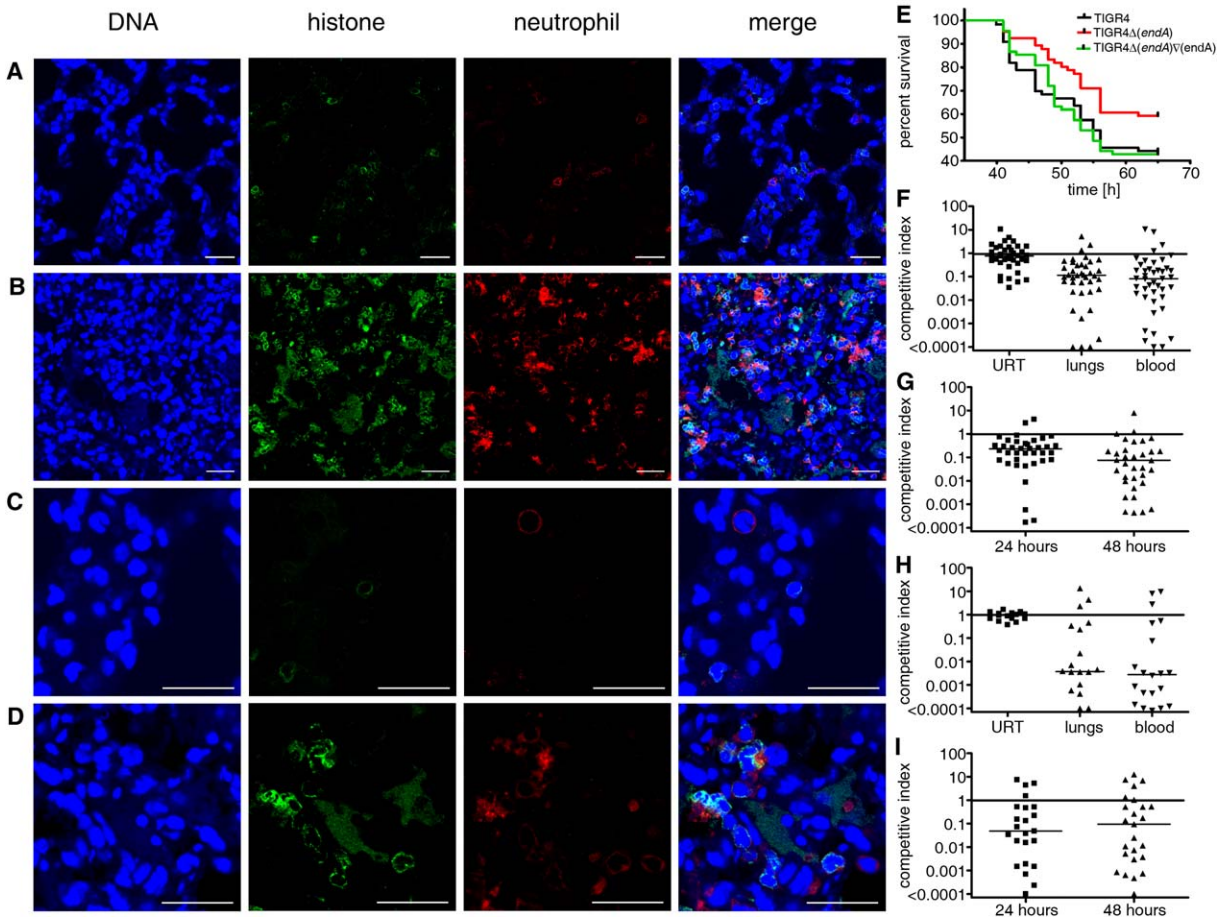

Figure 4. NETs Are Formed in Murine Pneumococcal Pneumonia and Play a Role in Defense against Pneumococci

(A–D) C57BL/6 mice were infected intranasally with either bacterial growth medium (A and C) or TIGR4 pneumococci (B and D). Forty-eight hours postinfection, the lungs were removed and stained for DNA (blue), histone H1 (green), and a neutrophil-specific surface marker (red). Scale bars represent 20 μm ([C] and [D] represent close-ups). Whereas in mock-infected controls (A and C), DNA and histone both are restricted to nuclei, in infected samples (B and D), extracellular DNA and histone can be observed, lining the alveoli. This shows that NETs are formed in murine pneumococcal pneumonia.

(E) C57BL/6 mice were infected intranasally with TIGR4, TIGR4Δ(*endA*), or TIGR4Δ(*endA*)∇(*endA*) pneumococci (n = 66). Two independent observers assessed the health status to assign pathology scores as described previously [8]. Mice infected with TIGR4Δ(*endA*) had a delayed onset of severe disease and a significantly higher survival rate than both TIGR4 and TIGR4Δ(*endA*)∇(*endA*) as determined by the Kaplan-Meier-analysis log-rank test (A p value < 0.05 was considered significant).

(F–I) C57BL/6 mice were infected intranasally with a 1:1 mix of TIGR4 and TIGR4Δ(*endA*) (F-G) or a 1:1 mix of TIGR4Δ(*endA*)∇(*endA*) and TIGR4Δ(*endA*) (H–I). Bacterial counts in the upper respiratory tract (URT), lungs, and bloodstream were determined when mice were sacrificed (F and H). Bacterial counts per ml blood were determined 24 hr and 48 hr postinfection (G and I). The competitive index shown is based on the ratio of number of bacteria recovered from the two strains. A competitive index of 1 indicates equal numbers of wild-type and mutant bacteria. Values lower than 1 indicate that the mutant is outcompeted. Each dot represents one mouse. Median values are indicated by horizontal bars. All strains are able to colonize the URT of mice to the same extent. However, TIGR4Δ(*endA*) is outcompeted by both TIGR4 (F–G) and TIGR4Δ(*endA*)∇(*endA*) (H–I) in the lungs and in the bloodstream. This shows that TIGR4Δ(*endA*) is defective in its invasion ability.

is inflammation with an abundant recruitment of neutrophils [2]. Pneumococci are typically expressing an antiphagocytic capsule, suggesting that phagocytosis early during infection might provide little protection to the host. Also, because the vast majority of pneumococcal infections result in nasopharyngeal colonization without further spread, there must be mechanisms operating in the host that confine the organism to its local site of infection. We therefore hypothesized that extracellular capture of pneumococci in NETs represents one such mechanism.

Here, we show that NETs trap pneumococci (Figure 1). This is in agreement with previous studies showing that NETs trap other microbes such as *Salmonella enterica* serovar Typhimurium, *Shigella flexneri*, *Staphylococcus aureus* [4], and *Candida albicans* [16]. In contrast to other microbes, pneumococci are not killed by NETs (Figure 1C). Yet, NET trapping of pneumococci could be important in restricting the dissemination of the bacteria. In pneumococcal infections, NETs might initially capture the bacteria, thus reducing their spread by confining bacteria to specific areas.

An efficient means to evade NETs would be to disintegrate the DNA backbone. EndA is a bacterial cell-associated endonuclease [7, 12] that allows pneumococci to efficiently degrade extracellular DNA and to escape

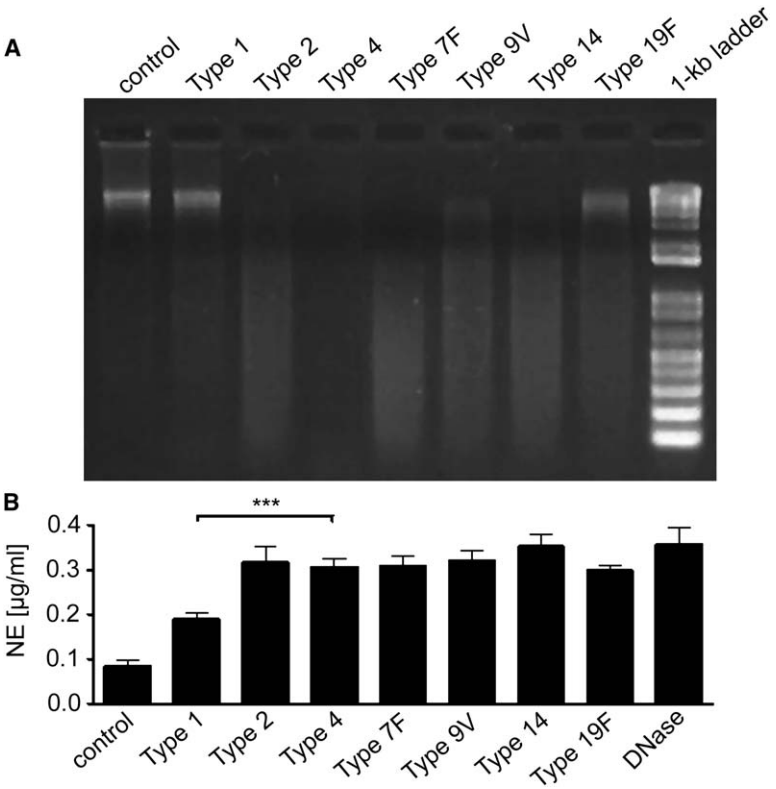

Figure 5. Most Pneumococcal Serotypes Show DNase Activity and Degrade NETs

(A) Salmon sperm DNA was incubated with pneumococcal strains of the indicated serotypes and separated on an agarose gel. A sample without pneumococci was used as negative control. Differences in DNase activity of the tested strains can be observed, with especially low DNase activity for the serotype 1 strain.

(B) NETs were treated with RPMI medium (control), pneumococcal strains of different serotypes, or bovine pancreatic DNase. The concentration of NE in the supernatant was measured as a reporter of NET degradation. Mean and SEM are shown. The assay was performed three times and analyzed with the nonparametric Mann-Whitney test. A p value < 0.05 was considered significant. Compared to TIGR4 (serotype 4), a significantly lower concentration of NE could be measured in neutrophils exposed to pneumococci of type 1. This is reflecting the type 1's low DNase activity and capability to degrade NETs.

from NETs by degrading their DNA backbone (Figures 2 and 3). Pneumococci lacking *endA* are not able to free themselves from NETs (Figure 3) and are also less virulent after intranasal challenge in mice (Figure 4). *endA* knockouts were outcompeted by the wild-type strain in the lungs and in the bloodstream but not in the upper respiratory tract, where initial colonization occurs (Figure 4). Because EndA is cell bound [7, 12], it is unable to rescue *endA* mutant bacteria from NETs in mixed infections. The cell association of EndA implies that it can only degrade NETs locally in the vicinity of trapped bacteria. This probably explains why NETs remain present in the lungs after infections with pneumococci expressing EndA.

Our data suggest a pivotal role for EndA in facilitating progression from localized pneumococcal infections in the upper airways into an invasive disease. Hence, our findings explain why *endA* was identified as a virulence gene by signature-tagged mutagenesis [17].

Pneumococcal strains that express EndA at high levels are likely to be able to escape NETs, making them more prone to cause an invasive disease. Even though most pneumococcal strains tested, all capable of causing invasive disease in humans, express EndA and destroy NETs, the enzyme levels produced might differ. In this study, a strain of type 1 (PJ1354), exhibited a significantly lower DNase activity compared to the other strains tested (Figure 5). It has recently been shown that serotype 1 isolates of this clonal type cause less severe invasive infections, compared to most other pneumococcal types, with a high proportion of pneumonia in humans [14]. Hence, differences in DNase activity might endow individual strains of this highly variable species with different risks of causing severe invasive disease in man.

Conclusions

This study presents a novel role for neutrophils in innate immune responses to bacterial infections. We show the presence of neutrophil extracellular traps (NETs) in pneumococcal pneumonia and that NETs are able to trap, but not kill, pneumococci. We further find that EndA, a pneumococcal nuclease, is able to degrade NETs. Our findings suggest that EndA acts as a virulence determinant counteracting host-mediated trapping by NETs, thereby promoting bacterial spread from local sites to the lungs and from the lungs to the bloodstream.

Supplemental Data

Supplemental Data include Supplemental Experimental Procedures and four figures and are available with this article online at: http://www.current-biology.com/cgi/content/full/16/4/401/DC1/.

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References

1. World Health Organization (2003). Pneumococcal vaccines. Weekly Epidemiological Record *14*, 110–119.
2. Kadioglu, A., and Andrew, P.W. (2004). The innate immune response to pneumococcal lung infection: The untold story. Trends Immunol. *25*, 143–149.
3. Hirsch, J.G. (1958). Bactericidal action of histone. J. Exp. Med. *108*, 925–944.
4. Brinkmann, V., Reichard, U., Goosmann, C., Fauler, B., Uhlemann, Y., Weiss, D.S., Weinrauch, Y., and Zychlinsky, A. (2004). Neutrophil extracellular traps kill bacteria. Science *303*, 1532–1535.
5. Sumby, P., Barbian, K.D., Gardner, D.J., Whitney, A.R., Welty, D.M., Long, R.D., Bailey, J.R., Parnell, M.J., Hoe, N.P., Adams, G.G., et al. (2005). Extracellular deoxyribonuclease made by group A Streptococcus assists pathogenesis by enhancing evasion of the innate immune response. Proc. Natl. Acad. Sci. USA *102*, 1679–1684.
6. Udou, T., and Ichikawa, Y. (1979). Characteristics of extracellular nuclease production in Staphylococcus aureus. Microbiol. Immunol. *23*, 679–684.
7. Puyet, A., Greenberg, B., and Lacks, S.A. (1990). Genetic and structural characterization of endA. A membrane-bound nuclease required for transformation of Streptococcus pneumoniae. J. Mol. Biol. *213*, 727–738.
8. Sandgren, A., Albiger, B., Orihuela, C.J., Tuomanen, E., Normark, S., and Henriques-Normark, B. (2005). Virulence in mice of pneumococcal clonal types with known invasive disease potential in humans. J. Infect. Dis. *192*, 791–800.
9. Brenner, S.L., and Korn, E.D. (1979). Substoichiometric concentrations of cytochalasin D inhibit actin polymerization. Additional evidence for an F-actin treadmill. J. Biol. Chem. *254*, 9982–9985.
10. Staali, L., Morgelin, M., Bjorck, L., and Tapper, H. (2003). Streptococcus pyogenes expressing M and M-like surface proteins are phagocytosed but survive inside human neutrophils. Cell. Microbiol. *5*, 253–265.
11. Tettelin, H., Nelson, K.E., Paulsen, I.T., Eisen, J.A., Read, T.D., Peterson, S., Heidelberg, J., DeBoy, R.T., Haft, D.H., Dodson, R.J., et al. (2001). Complete genome sequence of a virulent isolate of Streptococcus pneumoniae. Science *293*, 498–506.
12. Lacks, S., and Neuberger, M. (1975). Membrane location of a deoxyribonuclease implicated in the genetic transformation of Diplococcus pneumoniae. J. Bacteriol. *124*, 1321–1329.
13. Hemsley, C., Joyce, E., Hava, D.L., Kawale, A., and Camilli, A. (2003). MgrA, an orthologue of Mga, Acts as a transcriptional repressor of the genes within the rlrA pathogenicity islet in Streptococcus pneumoniae. J. Bacteriol. *185*, 6640–6647.
14. Sjostrom, K., Spindler, C., Ortqvist, A., Kalin, M., Sandgren, A., Kuhlmann-Berenzon, S., and Henriques-Normark, B. (2006). Clonal and capsular types decide whether pneumococci will act as a primary or opportunistic pathogen. Clin. Infect. Dis. *42*, 451–459.
15. Ortqvist, A., Kalin, M., Julander, I., and Mufson, M.A. (1993). Deaths in bacteremic pneumococcal pneumonia. A comparison of two populations–Huntington, WVa, and Stockholm, Sweden. Chest *103*, 710–716.
16. Urban, C.F., Reichard, U., Brinkmann, V., and Zychlinsky, A. (2006). Neutrophil extracellular traps capture and kill Candida albicans yeast and hyphal forms. Cell. Microbiol., in press.
17. Hava, D.L., and Camilli, A. (2002). Large-scale identification of serotype 4 Streptococcus pneumoniae virulence factors. Mol. Microbiol. *45*, 1389–1406.