This article contains data related to the inflammatory cytokine and investigated pathways involved in bacterial clearance reported in "Airway infection with Nontypeable Haemophilus influenzae is more rapidly eradicated in vitamin D deficient mice" (Serré et al., 2018) [1]. Vitamin D deficient or sufficient mice were oropharyngeally instilled with $10^6$ NTHi and sacrificed at 4, 8, 24 and 72 h post-infection. We measured proinflammatory cytokines (KC, TNF-α, IL-1β, IL6 and MCP-1) markers of bacterial clearance pathways (myeloid peroxidase, nitric oxide, complement C5a and immunoglobulin A) in bronchoalveolar fluid (BALF) during infection and mRNA expression levels of innate immune defense mechanism markers (mucin glycoproteins, pathogen recognitions receptor TLR2 and TLR4, antimicrobial peptides SLPI, REG3γ, lysozyme, BD-1, BD-2,
BD-3 and surfactant proteins SP-A and SP-D) in lung homogenate. Finally, genomic DNA of NTHi (protein D) measured in lung homogenate was used as an indicator of NTHi invasion of alveolar macrophages or epithelial cells.

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Specifications table

| Subject area          | Biology        |
|-----------------------|----------------|
| More specific subject area | Immunology   |
| Type of data          | Tables and Figures |
| How data was acquired | Multiplex Elisa, qRT-PCR |
| Data format           | Analyzed       |
| Experimental factors  | Broncho-alveolar lavage fluid and lung homogenate obtained after in vivo exposure to cigarette smoke and/or vitamin D deficiency post-infection with NTHi |
| Experimental features | mRNA and protein expression of inflammatory cytokines and key markers of bacterial clearance pathways |
| Data source location  | Leuven, Belgium |
| Data accessibility    | Within this article |
| Related research article | Jef Serré, Carolien Mathyssen, Tom Tanjeko Ajime, Hannelie Korf, Karen Maes, Nele Heulens, Conny Gysemans, Chantal Mathieu, Bart Vanaudenaerde, Wim Janssens, Ghislaine Gayan-Ramirez; Airway infection with Nontypeable Haemophilus influenzae is more rapidly eradicated in vitamin D deficient mice; the journal of steroid biochemistry and molecular biology, available online November 2018, In Press, [1]. |

Value of the data

- Model to assess bacterial clearance during infection to compare with other bacteria.
- This dataset provides more insight in the inflammatory cytokine response during an infection with NTHi.
- This dataset shows the activation of multiple antimicrobial pathways.
- A protocol for the detection of chromosomal bacterial DNA of NTHi in lung homogenate.

1. Data

The data in this article acts as supplementary analysis that were performed in [1]. Table 1 summarizes the levels of pro-inflammatory cytokines measured in BALF before and after infection with NTHi in both groups. Table 2 shows the primer sequences of the studied genes while Table 3 reports mRNA expression levels of these genes per group during and after infection. Myeloperoxidase and nitric oxide production in BALF for both groups are given in Table 4. Fig. 1 depicts protein levels of complement C5a and immunoglobulin A in BALF during infection in each group. Chromosomal DNA concentration of NTHi in lung homogenate measured over time is shown on Fig. 2 for both groups.
Table 1
Faster resolution of pro-inflammatory mediators in vitamin D deficient mice compared to vitamin D sufficient mice. KC, TNF-α, IL-6, IL-1β and MCP-1 was determined in BALF in sufficient and vitamin D deficient mice (n = 6 mice/group/time point). Data are presented in pg/ml.

| Target (pg/ml) | DIET   | Baseline | Post-infection |
|---------------|--------|----------|----------------|
|               |        |          | 4 h            | 8 h            | 24 h           | 72 h           |
| KC            | Sufficient | 6.56 ± 1.36 | 7199 ± 1466 | 2243 ± 1004 | 37.66 ± 28.07 | 24.21 ± 8.33 |
|               | Deficient | 6.33 ± 2.42 | 7443 ± 991.5 | 1769 ± 1295 | 32.69 ± 17.68 | 26.45 ± 12.22 |
| TNF-α         | Sufficient | 5.57 ± 1.22 | 6127 ± 274.9 | 5455 ± 612.8 | 517 ± 130.9   | 112.2 ± 34.89 |
|               | Deficient | 3.25 ± 1.37 | 6083 ± 318.7 | 4303 ± 1084 | 478.1 ± 248.1 | 34.89 ± 14.89 |
| IL-6          | Sufficient | 2.82 ± 2.48 | 26214 ± 7857 | 27908 ± 5129 | 4257 ± 2240  | 270.2 ± 139.1 |
|               | Deficient | 1.84 ± 0.99 | 28356 ± 5708 | 20300 ± 6397 | 2740 ± 1860  | 49.88 ± 20.16 |
| IL-1β         | Sufficient | 0.22 ± 0.30 | 13.15 ± 7.40 | 48.17 ± 15.56 | 17.27 ± 12.18 | 2.90 ± 2.41  |
|               | Deficient | 0.07 ± 0.10 | 12.41 ± 5.49 | 34.97 ± 12.50 | 17.20 ± 8.75  | 1.34 ± 1.09  |
| MCP-1         | Sufficient | /         | 103.3 ± 58.33 | 32.74 ± 20.57 | 178.1 ± 111.7 | 25.88 ± 11.56 |
|               | Deficient | /         | 84.52 ± 35.11 | 24.66 ± 6.43  | 83.97 ± 32.70 | 27.87 ± 11.57 |

Data are expressed as mean ± SD.

* p < 0.05.

** p < 0.01 (Mann-Whitney test, sufficient vs deficient).

Table 2
Primer sequences of the house keeping gene (RPL27), mucin glycoproteins (MUC5AC, MUC5B), a pathogen recognition receptor (TLR2, TLR4), the antimicrobial peptides (SLPI, Lysozyme, REG3γ, CRAMP, BD 1–3), the surfactant proteins (SP-A, SP-D), the tissue inhibitor of matrix metalloproteinase (TIMP1) and the matrix metalloproteinases (MMP9 and MMP12). Primer efficiency is in the range of 90–105%.

| Target | Forward primer | Reverse primer | Accession ID |
|--------|----------------|----------------|--------------|
| RPL27  | 5'-GTCGAGATGGGCAAGTTCT-3' | 5'-TTCTTCAGATGACGGCTT-3' | NM_011289.3 |
| MUC5AC | 5'-CTGTAACACCCACGTCCTAAG-3' | 5'-AGGGCTGGAAGCTAGTAAAG-3' | NM_010844.1 |
| MUC5B  | 5'-CCGTTCTCTTCTCCCAATATC-3' | 5'-TCTAGTCGTTCGTGTTCTTCA-3' | NM_028801.2 |
| TLR2   | 5'-CACTATCCGGAGGTGATCATATC-3' | 5'-GGGAAACCTCTGCTTCTTAC-3' | NM_01905.3  |
| TLR4   | 5'-GGGTATTGACACCCCTGTTCTAT-3' | 5'-CAAGATGTGACTGGAAATACAG-3' | NM_0212973.3 |
| BD-1   | 5'-AGCAAGGTGTGCCATTTCT-3' | 5'-GCTATCTCCGGAGTTCACC-3' | NM_007843.3  |
| BD-2   | 5'-GGCAGGAGACTCTCTCTGCTT-3' | 5'-GGCAAAATGGCTGCAGACA-3' | NM_010030.1  |
| BD-3   | 5'-GTGCTAGAGCTCAGTTATTCAC-3' | 5'-CCGGATCTCTTTGCTCTATT-3' | NM_013756.2  |
| SLPI   | 5'-CCTGCTAGGGGATATTTGCGAA-3' | 5'-CCGCAATCTACGGAGTAGC-3' | NM_01414.3  |
| Lysozyme | 5'-ATGGAATGGCCGTGGCTACAT-3' | 5'-GGGCCACGGTGTAGATT-3' | NM_017372.3  |
| REG3γ  | 5'-TGGCTATGTTTCTCTATTT-3' | 5'-CACTCCCATCCACTCTTGT-3' | NM_012610.3  |
| CRAMP  | 5'-ACCAATCTACTGCTCTCT-3' | 5'-CTCTGCCACCCACATAC-3' | NM_009921.2  |
| SP-A   | 5'-GGCATAACCAACTGCTCTCT-3' | 5'-GCTACACCCGAAAGACAGC-3' | NM_023134.4  |
| SP-D   | 5'-AAGGCTGCTTCTTACTGATG-3' | 5'-CCTGGACCAAAATTAGAAGAC-3' | NM_009160.2  |
| TIMP1  | 5'-GTGGGAAATGCGCGAGAT-3' | 5'-GGGCATATCCACAGGCCATT-3' | NM_01593.2   |
| MMP9   | 5'-TCCTCGAGGGAACCTTCTA-3' | 5'-TCTGGCTCTCCCATATCCT-3' | NM_013599.4  |
| MMP12  | 5'-TTTGTATGGCAGAGTTGAGGTA-3' | 5'-GCTCCATCAAAATGCGCAGAT-3' | NM_008605.3  |

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mRNA expression of antimicrobial pathway markers are similarly enhanced in both group with infection except for B-defensins that could not be detected. Mucin glycoproteins (MUC5AC, MUC5B), a pathogen recognition receptor (TLR2, TLR4), the antimicrobial peptides (SLPI, Lysozyme, REG3γ, CRAMP, BD 1–3), surfactant proteins (SP-A, SP-D) were determined in lung homogenate (n = 6 mice/group/time point). Data are expressed as fold change relative to RPL27 as mean ± SD.

| Target | DIET          | Baseline      | Post-infection |
|--------|---------------|---------------|----------------|
|        |               | 4 h           | 8 h            | 24 h          | 72 h          |
| MUC5AC | Sufficient    | 1.39 ± 0.71   | 2.13 ± 0.83    | 1.35 ± 0.48   | 2.13 ± 1.20   |
|        | Deficient     | 0.85 ± 0.60   | 1.87 ± 1.02    | 1.60 ± 0.79   | 2.15 ± 1.11   |
| MUC5B  | Sufficient    | 1.37 ± 0.71   | 2.07 ± 0.67    | 0.84 ± 0.19   | 1.67 ± 1.40   |
|        | Deficient     | 1.16 ± 0.63   | 1.85 ± 0.96    | 0.87 ± 0.46   | 1.92 ± 1.09   |
| TLR2   | Sufficient    | 1.16 ± 0.70   | 7.98 ± 3.16    | 10.79 ± 3.51  | 5.40 ± 2.00   |
|        | Deficient     | 1.39 ± 0.37   | 10.29 ± 6.51   | 10.72 ± 4.27  | 6.09 ± 3.42   |
| TLR4   | Sufficient    | 2.22 ± 1.32   | 5.21 ± 2.47    | 5.87 ± 2.81   | 4.55 ± 2.18   |
|        | Deficient     | 3.47 ± 0.33   | 6.35 ± 4.87    | 7.11 ± 4.08   | 6.07 ± 3.54   |
| BD-1   | No detection in lung homogenate with 3 different primers |    |                |               |               |
| BD-2   | No detection in lung homogenate with 3 different primers |    |                |               |               |
| BD-3   | No detection in lung homogenate with 3 different primers |    |                |               |               |
| REG3g  | Sufficient    | 1.14 ± 0.63   | 2.64 ± 1.69    | 1.43 ± 0.99   | 5.13 ± 4.24   |
|        | Deficient     | 0.83 ± 0.37   | 1.81 ± 1.15    | 2.02 ± 1.05   | 4.87 ± 3.74   |
| SLPI   | Sufficient    | 1.18 ± 0.88   | 3.75 ± 2.52    | 6.78 ± 4.79   | 3.70 ± 3.05   |
|        | Deficient     | 1.00 ± 0.59   | 4.14 ± 2.37    | 6.25 ± 4.98   | 2.73 ± 2.17   |
| Lysozyme | Sufficient  | 1.19 ± 0.76   | 1.37 ± 0.65    | 0.98 ± 0.46   | 1.80 ± 1.19   |
|        | Deficient     | 1.92 ± 0.70   | 1.40 ± 0.56    | 1.63 ± 0.74   | 1.99 ± 1.03   |
| SP-D   | Sufficient    | 1.13 ± 0.67   | 1.51 ± 0.46    | 2.25 ± 0.84   | 1.88 ± 0.79   |
|        | Deficient     | 2.05 ± 1.06   | 1.94 ± 1.08    | 2.26 ± 0.65   | 1.91 ± 0.83   |
| SP-A   | Sufficient    | 1.06 ± 0.50   | 0.72 ± 0.53    | 0.39 ± 0.26   | 0.47 ± 0.34   |
|        | Deficient     | 1.32 ± 0.57   | 0.75 ± 0.51    | 0.39 ± 0.26   | 0.46 ± 0.21   |

** p < 0.01 (Mann-Whitney test, sufficient vs deficient).

2. Experimental design, materials and methods

C57BL/6jolaH mice (3 weeks old) were made vitamin D deficient or sufficient through the use of a vitamin D deficient or a standard control diet respectively [1]. Series 2 of [1] is elaborated in more detail below. Briefly, mice were infected with NTHi at 9 weeks of age and sacrificed at baseline and 4, 8, 24 and 72 h post-infection (n = 6 mice/group/time point).

2.1. Pro-inflammatory mediators in the bronchoalveolar lavage

Pro-inflammatory cytokines KC, Tumor Necrosis Factor (TNF)-α, Monocyte Chemotactic Protein (MCP)-1, Interleukin (IL)-6 and IL-1β were measured in the BAL fluid as described in [1].

2.2. mRNA expression levels of inflammatory mediators, antimicrobial markers and matrix metalloproteinases (MMPs)

Total RNA was extracted, converted to cDNA and analyzed via qRT-PCR as described in [1]. Primer sequences (Table 2) and mRNA expression levels (Table 3) of mucin glycoproteins (MUC), pattern recognition receptors (PRR), antimicrobial peptides (AMP) and surfactant proteins (SP): MUC5AC, MUC5B, toll-like receptor (TLR) 2, TLR4, Lysozyme, secreted leukocyte protease inhibitor (SLPI), β-defensin 1, 2, 3 and cathelicidin related antimicrobial peptide (CRAMP), regenerating islet-derived protein 3-gamma (REG3γ), SP-A and D used in [1] are given below.
3. Myeloperoxidase and nitric oxide production in BALF

Myeloperoxidase (MPO) levels were measured in BALF as described in [1]. Primary metabolite of nitric oxide, nitrite (NO$_2^-$), was measured via Gries reagent kit (Thermo Fischer scientific) according to manufacturer's instructions.

### Table 4
Myeloperoxidase and Nitric oxide protein levels were elevated during NTHi infection. MPO and NO were measured in BALF at baseline and at 4, 8, 24 and 72 h post-infection (n = 6 mice/group/time point). Data are expressed in pg/ml MPO and μM NO$_2^-$ as mean ± SD.

| Target       | DIET       | Baseline | Post-infection |
|--------------|------------|----------|----------------|
|              |            |          | 4h  | 8h  | 24h  | 72h  |
| MPO (pg/ml)  | Sufficient | 1.45 ± 0.22 | 163.4 ± 176.2 | 384.3 ± 41.96 | 303.1 ± 101.2 | 148.2 ± 12.49 |
|              | Deficient  | 1.32 ± 0.17 | 103.8 ± 53.89 | 320.2 ± 95.74 | 295.0 ± 83.91 | 137.6 ± 30.50 |
| NO$_2^-$ (μM) | Sufficient | 0.91 ± 0.54 | 1.26 ± 0.50 | 1.07 ± 0.60 | 1.61 ± 0.75 | 1.77 ± 0.44 |
|              | Deficient  | 0.99 ± 0.13 | 1.65 ± 0.24 | 1.34 ± 0.50 | 1.30 ± 0.33 | 1.05 ± 0.48 (*) |

* p < 0.05 (Mann-Whitney test, sufficient vs deficient).

Fig. 1. Complement C5a and Immunoglobulin A (IgA) protein levels are enhanced to the same extent in both group during the infection period. Complement C5a and IgA protein levels were measured in BALF during infection (n = 6 mice/group/time point). Data are expressed in pg/ml C5a and ng/ml IgA as mean ± SD.

### 3. Myeloperoxidase and nitric oxide production in BALF

Myeloperoxidase (MPO) levels were measured in BALF as described in [1]. Primary metabolite of nitric oxide, nitrite (NO$_2^-$), was measured via Gries reagent kit (Thermo Fischer scientific) according to manufacturer’s instructions.

#### 3.1. Complement C5a and Immunoglobulin A measurement in BALF

Complement C5a was measured in 1:100 diluted BALF, using a coated Complement C5a Mouse ELISA kit (ab193718, Abcam, Cambridge) with a range of 1.64–400 pg/ml. Immunoglobulin A (IgA) levels in BALF were measured using an IgA mouse Uncoated ELISA kit (ThermoFisher Scientific, Massachusetts) with a range of 0.39–25 ng/ml. ELISA’s were performed according to manufacturer’s instructions.
3.2. NTHi genomic DNA quantification

Genomic DNA (gDNA) was extracted out of 20–25 mg lung tissue using PureLink Genomic DNA Kit (Invitrogen). Briefly, lung tissue was homogenized in 200 μl genomic digestion buffer with Proteinase K (2 mg/ml) and incubated at 56 °C overnight. Particulate material was removed by centrifugation at 12,000 g for 3 min. RNA was broken down by adding RNase A and incubating it at room temperature for 2 min. Column steps were executed according to manufacturers instructions and gDNA was eluted in 50 μl elution buffer (10 mM Tris–HCl, pH 8.0). qPCR was performed using 160 ng gDNA, 300 nM/primer, 1 mM MgCl, 1 x KAPA SYBR® FAST master mix and 1x High rox (included in KAPA SYBR® FAST qPCR kit master mix (2 x ) universal, KAPAbiosystems, Boston) for one reaction. Primer set was designed for the protein D sequence on gDNA of NTHi: FOR- 5’GGCCCAGGTTGGTATAGTTAG3’, REV- 5’TCGGGCAGTGCATCTTTAC3’. Concentration gDNA of NTHi was quantified by using a 1:10 standard dilution curve with range of 65,200–0.652 pg of pure gDNA from freshly grown NTHi.

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Transparency document. Supporting information

Transparency data associated with this article can be found in the online version at https://doi.org/10.1016/j.dib.2018.12.048.

Reference

[1] J. Serré, C. Mathyssen, T. Tanjeko Ajime, H. Korf, K. Maes, N. Heulens, C. Gysemans, C. Mathieu, B.M. Vanaudenaerde, W. Janssens, G. Gayan-Ramirez, Airway infection with Nontypeable Haemophilus influenzae is more rapidly eradicated in vitamin D deficient mice, J. Steroid Biochem. Mol. Biol. (2018).