

Analysis

2025-12-08

Background

This project focuses on cytokines and their role in controlling viral replication within the epithelium.

Methods

Fold changes are computed from actin.

When data are paired, paired t tests are used. When data are not paired, both unpaired t tests and linear mixed models (lmm) are used to analyze the data. t tests first average the outcome in the replicates, lmm uses all data. Lmm may be more efficient, but when there are warnings about singular values, the validity of their results is in doubt.

Fig 5A

Data is log10 transformed.

Question: Is there a statistically significant difference between the treated group and its associated untreated control?

For Keratinocyte, there are 10 donors; for Fibroblast, there are 4 donors.

Table 1: Paired t test p values.

	Keratinocyte	Fibroblast
CXCL10	<0.001	0.001
CCL5	0.023	0.885
IL15	<0.001	0.002
IL12A	<0.001	0.006
IFI16	<0.001	0.005

Treated versus untreated is highly significant except for CCL5 on fibroblasts.

Question: Is there a statistically significant difference between treated keratinocytes and treated fibroblasts

Table 2: Paired t test p values.

	treated	untreated
CXCL10	0.033	0.402
CCL5	0.461	0.406
IL15	0.049	0.465
IL12A	0.153	0.381
IFI16	0.183	0.392

Treated keratinocytes versus treated fibroblasts is highly significant except for CCL5. Untreated keratinocytes versus untreated fibroblasts is not significant for any cytokine.

Fig 5B

Data is not log10 transformed.

Question: Is there a statistically significant difference between keratinocytes (orange) and fibroblasts (blue) at each dose in each cytokine of interest?

Table 3: Paired t test p values.

	0	0.1	1	10	100
CXCL10	0.129	0.043	0.033	0.013	0.018
CCL5	0.263	0.276	0.337	0.110	0.169
IL15	0.028	0.916	0.844	0.977	0.986
IL12A	0.010	<0.001	<0.001	0.009	0.010
IFI16	0.716	0.126	0.257	0.658	0.906

Question: For each tissue, is there a statistically significant difference between each dose and the next lower dose? IE is there a difference between 0.1 and 1, 1 and 10, 10 and 100? The goal of this question is to establish what dose is the peak response

Table 4: Paired t test p values.

	0.1 vs. 0	1 vs. 0.1	10 vs. 1	100 vs. 10
CXCL10, Keratinocytes	<0.001	<0.001	0.004	0.280
CXCL10, Fibroblasts	0.051	0.020	0.006	0.242
CCL5, Keratinocytes	0.427	0.075	0.406	0.293
CCL5, Fibroblasts	0.153	0.896	0.311	0.129
IL15, Keratinocytes	<0.001	<0.001	0.047	0.812
IL15, Fibroblasts	0.002	0.019	0.053	0.454
IL12A, Keratinocytes	0.004	<0.001	0.594	0.216
IL12A, Fibroblasts	0.048	0.032	0.429	0.273
IFI16, Keratinocytes	0.026	<0.001	0.708	0.569
IFI16, Fibroblasts	0.101	0.072	0.045	0.692

Fig 5C

Data is log10 transformed.

Question: Is there a statistically significant difference between keratinocytes (orange) and fibroblasts (blue) at each dose in each cytokine of interest?

Table 5: Paired t test p values.

	0	0.1	1	10	100
CXCL10	0.402	0.030	0.033	0.006	0.005
CCL5	0.406	0.293	0.461	0.277	0.780
IL15	0.465	0.007	0.049	0.008	0.002
IL12A	0.381	0.124	0.153	0.020	0.012
IFI16	0.393	0.744	0.473	0.572	0.140

Question: For each tissue, is there a statistically significant difference between each dose and the next lower dose? The goal of this question is to establish what dose is the peak response

Table 6: Paired t test p values.

	0.1 vs. 0	1 vs. 0.1	10 vs. 1	100 vs. 10
CXCL10, Keratinocytes	<0.001	<0.001	0.004	0.279
CXCL10, Fibroblasts	0.051	0.020	0.006	0.243
CCL5, Keratinocytes	0.433	0.075	0.406	0.294
CCL5, Fibroblasts	0.147	0.899	0.309	0.126
IL15, Keratinocytes	<0.001	<0.001	0.047	0.817
IL15, Fibroblasts	0.002	0.019	0.054	0.455
IL12A, Keratinocytes	0.003	<0.001	0.709	0.146
IL12A, Fibroblasts	0.047	0.032	0.428	0.268
IFI16, Keratinocytes	0.144	<0.001	0.633	0.928
IFI16, Fibroblasts	0.102	0.071	0.044	0.685

Fig 5D

Data is log10 transformed.

Question: Is there a statistically significant difference between keratinocytes (orange) and fibroblasts (blue) at each time point for each cytokine individually?

Table 7: Paired t test p values.

	Untreated	4HPT	24HPT
CXCL10	0.402	0.005	0.013
CCL5	0.406	0.780	0.565
IL15	0.465	0.002	0.882
IL12A	0.381	0.012	0.696
IFI16	0.392	0.154	0.693

Question: For fibroblasts, is there a statistically significant difference between untreated and 4HPT for each cytokine? Is there a statistically significant difference between 4HPT and 24HPT for each cytokine?

Table 8: Paired t test p values.

	4HPT vs. Untreated	24HPT vs. 4HPT
CXCL10, Keratinocytes	<0.001	<0.001
CXCL10, Fibroblasts	<0.001	0.011
CCL5, Keratinocytes	0.992	<0.001
CCL5, Fibroblasts	0.078	0.117
IL15, Keratinocytes	<0.001	0.250
IL15, Fibroblasts	0.002	0.040
IL12A, Keratinocytes	<0.001	0.411

IL12A, Fibroblasts	<0.001	0.107
IFI16, Keratinocytes	<0.001	0.584
IFI16, Fibroblasts	0.001	0.366

Fig 4C

Data is not log10 transformed.

Question: Compare the IC50 value between asymptomatic and severe donors. Data represents three independent biological replicates for each donor

Table 9: Linear mixed model.

	IC50
severe	0.59 (CI=-3.89,5.07, p=0.797)

Fig 4D

Data is not log10 transformed.

Question: Compare the expression levels of both genes between asymptomatic and severe donors. Data represents three independent biological replicates for each donor

Table 10: Linear mixed model.

IFNGR1	
severe	0.25 (CI=-0.32, 0.82, p=0.393)

Table 11: Linear mixed model.

STAT1	
severe	0.02 (CI=-0.48,0.51, p=0.949)

Fig 6A, 6B, and 6C

Data is log10 transformed for 6B and 6C and not 6A.

Question: Determine if there is a statistically significant difference between asymptomatic and severe donors at each dose (6A, 6B) or at each time (6C) for each gene (CXCL10, CCL5, IL15, IL12, IFI16) at dose 100.

Data represents three independent biological replicates for all 10 donors.

We first analyzed the data with linear mixed models. There are some warnings about singular values in 6B and 6C. Hence we re-analyzed the data with unpaired t tests.

Table 12: Linear mixed models.

	6A	0	0.1	1	10	100
CXCL10	0.81 (CI=-0.98, 2.60, p=0.373)	5.42 (CI=1.11,9.72, p=0.014)*	4.71 (CI= 0.61,8.81, p=0.024)*	3.07 (CI= 0.35,5.79, p=0.027)*	1.83 (CI=-0.36,4.02, p=0.102)	
CCL5	-0.30 (CI=-1.28, 0.69, p=0.554)	0.32 (CI=-1.07, 1.71, p=0.653)	0.49 (CI=-0.64, 1.62, p=0.396)	0.37 (CI=-1.25, 2.00, p=0.655)	-0.21 (CI=-1.20, 0.79, p=0.685)	
IL15	1.20 (CI=-0.89, 3.29, p=0.261)	1.98 (CI=-1.10,5.05, p=0.208)	2.20 (CI=-0.86,5.26, p=0.158)	1.50 (CI=-0.88,3.87, p=0.217)	1.18 (CI=-0.86,3.21, p=0.256)	
IL12A	0.45 (CI=-1.44, 2.35, p=0.640)	1.64 (CI=-1.26, 4.55, p=0.268)	1.62 (CI=-1.07, 4.31, p=0.238)	0.93 (CI=-1.54, 3.39, p=0.460)	0.65 (CI=-1.24, 2.55, p=0.498)	
IFI16	0.41 (CI=-0.94,1.76, p=0.551)	1.14 (CI=-0.98,3.26, p=0.293)	1.19 (CI=-0.49,2.86, p=0.165)	1.28 (CI=-0.83,3.39, p=0.234)	0.54 (CI=-1.01,2.09, p=0.499)	

Table 13: Unpaired t tests.

	6A	0	0.1	1	10	100
CXCL10	0.400	0.047	0.074	0.090	0.163	
CCL5	0.571	0.669	0.422	0.670	0.695	
IL15	0.302	0.268	0.226	0.278	0.317	
IL12A	0.657	0.322	0.296	0.493	0.525	
IFI16	0.578	0.349	0.233	0.299	0.530	

```

## boundary (singular) fit: see help('isSingular')

```

Table 14: Linear mixed models.

6B	0	0.1	1	10	100
CXCL10	0.00 (CI=-0.12,0.12, p=0.999)W	-1.39 (CI=-2.37,-0.40, p=0.006)**	-1.17 (CI=-2.07,-0.28, p=0.010)*	-0.68 (CI=-1.21,-0.14, p=0.013)*	-0.30 (CI=-0.81,0.20, p=0.238)
CCL5	0.00 (CI=-0.15,0.15, p=0.999)W	-0.19 (CI=-0.46,0.09, p=0.183)	-0.24 (CI=-0.42,-0.06, p=0.009)**W	-0.20 (CI=-0.58,0.18, p=0.302)	-0.03 (CI=-0.23,0.17, p=0.789)W
IL15	0.01 (CI=-0.09,0.11, p=0.808)W	-0.22 (CI=-0.80,0.35, p=0.450)	-0.29 (CI=-0.89,0.31, p=0.344)	-0.08 (CI=-0.41,0.26, p=0.653)	0.02 (CI=-0.30,0.34, p=0.910)
IL12A	0.01 (CI=-0.10,0.13, p=0.835)W	-0.35 (CI=-0.78,0.09, p=0.119)	-0.34 (CI=-0.69,0.01, p=0.058)	-0.13 (CI=-0.35,0.09, p=0.238)	-0.05 (CI=-0.19,0.09, p=0.495)W
IFI16	0.00 (CI=-0.11,0.10, p=0.990)W	-0.22 (CI=-0.56,0.12, p=0.201)	-0.23 (CI=-0.49,0.02, p=0.076)	-0.26 (CI=-0.58,0.06, p=0.108)	-0.04 (CI=-0.21,0.14, p=0.670)

Table 15: Unpaired t tests.

6B	0	0.1	1	10	100
CXCL10	0.893	0.029	0.042	0.040	0.273
CCL5	0.892	0.237	0.036	0.336	0.779
IL15	0.366	0.475	0.378	0.667	0.913
IL12A	0.362	0.167	0.106	0.295	0.422
IFI16	0.039	0.260	0.116	0.163	0.687

```

## boundary (singular) fit: see help('isSingular')

```

Table 16: Linear mixed models.

6C	Untreated	4HPT	24HPT
CXCL10	0.00 (CI=-0.12,0.12, p=0.999)W	-0.30 (CI=-0.81,0.20, p=0.238)	0.26 (CI=-0.37,0.90, p=0.415)
CCL5	0.00 (CI=-0.15,0.15, p=0.999)W	-0.03 (CI=-0.23,0.17, p=0.789)W	-0.20 (CI=-0.78,0.39, p=0.507)
IL15	0.01 (CI=-0.09,0.11, p=0.808)W	0.02 (CI=-0.30,0.34, p=0.910)	-0.02 (CI=-0.50,0.47, p=0.946)
IL12A	0.01 (CI=-0.10,0.13, p=0.835)W	-0.05 (CI=-0.19,0.09, p=0.495)W	-0.10 (CI=-0.46,0.26, p=0.597)
IFI16	0.00 (CI=-0.11,0.10, p=0.990)W	-0.04 (CI=-0.21,0.14, p=0.670)	-0.03 (CI=-0.37,0.30, p=0.853)

Table 17: Unpaired t tests.

6C	Untreated	4HPT	24HPT
CXCL10	0.893	0.273	0.452
CCL5	0.892	0.779	0.527
IL15	0.366	0.913	0.948
IL12A	0.362	0.422	0.618
IFI16	0.039	0.687	0.859

Fig 7A and 7B

Question: Determine if there is a statistically significant difference between the expression level of each gene (CXCL10, CCL5, IL15, IL12, IFI16) at each dose compared to the previous dose. For clarity, this is comparing 0.0 to 0.1, 0.1 to 1, 1 to 10, and 10 to 100. Data represents three independent biological replicates from 10 different donors

Data is log10 transformed.

Table 18: Paired t tests.

7A	0.1 vs. 0	1 vs. 0.1	10 vs. 1	100 vs. 10
CXCL9	0.001	<0.001	0.007	0.003
CXCL10	<0.001	<0.001	0.004	0.279
CXCL11	0.001	<0.001	0.003	0.008

Question: Determine if there is a statistically significant difference between asymptomatic and severe donors at each dose (0, 0.1, 1, 10, 100). Data represents three independent biological replicates for all 10 donors.

Data is log10 transformed.

```
## boundary (singular) fit: see help('isSingular')
## boundary (singular) fit: see help('isSingular')
## boundary (singular) fit: see help('isSingular')
```

Table 19: Linear mixed models.

7B	0	0.1	1	10	100
CXCL9	0.00 (CI=-0.29,0.29, p=1.000)W	-1.65 (CI=-2.84,-0.46, p=0.007)**	-1.30 (CI=-2.33,-0.28, p=0.013)*	-0.88 (CI=-1.34,-0.43, p=<0.001)**	-0.65 (CI=-1.08,-0.21, p=0.004)**
CXCL10	0.00 (CI=-0.12,0.12, p=0.999)W	-1.39 (CI=-2.37,-0.40, p=0.006)**	-1.17 (CI=-2.07,-0.28, p=0.010)*	-0.68 (CI=-1.21,-0.14, p=0.013)*	-0.30 (CI=-0.81,0.20, p=0.238)
CXCL11	0.00 (CI=-0.22,0.22, p=1.000)W	-1.31 (CI=-2.53,-0.08, p=0.037)*	-1.27 (CI=-2.29,-0.25, p=0.015)*	-0.62 (CI=-1.21,-0.04, p=0.036)*	-0.37 (CI=-1.03,0.28, p=0.264)

Table 20: Unpaired t tests.

7B	0	0.1	1	10	100
CXCL9	0.441	0.029	0.041	0.005	0.024
CXCL10	0.893	0.029	0.042	0.040	0.273
CXCL11	0.850	0.080	0.053	0.072	0.297

Fig 8

Data is log10 transformed.

Question: Determine if there is a difference in pg/mL of protein between asymptomatic and severe in each treatment group (mock, 100U, virus, 100U+virus).

```
## boundary (singular) fit: see help('isSingular')
## boundary (singular) fit: see help('isSingular')
```

Table 21: Linear mixed models.

	Mock	100U	Virus	Virus+100U
CXCL9 4HPT	all values identical	all values identical	all values identical	all values identical
CXCL9 12HPT	all values identical	-0.07 (CI=-0.17,0.02, p=0.146)	all values identical	-0.02 (CI=-0.07,0.02, p=0.317)
CXCL10 4HPT	all values identical	-0.30 (CI=-0.57,-0.02, p=0.033)*	all values identical	-0.24 (CI=-0.47,0.00, p=0.050)*
CXCL10 12HPT	all values identical	-0.49 (CI=-0.75,-0.24, p=<0.001)**W	all values identical	-0.48 (CI=-0.78,-0.18, p=0.002)**W

Table 22: Unpaired t test pvals.

	Mock	100U	Virus	Virus+100U
CXCL9 4HPT	all values identical	all values identical	all values identical	all values identical
CXCL9 12HPT	all values identical	0.220	all values identical	0.374
CXCL10 4HPT	all values identical	0.087	all values identical	0.110
CXCL10 12HPT	all values identical	0.004	all values identical	0.002

Question: Determine if there is a difference in pg/mL of protein between Mock and each treatment (100U, Virus, 100U+Virus) within each group (asymptomatic, severe). For example, if there is a statistical difference between asymptomatic mock vs asymptomatic 100U

Table 23: Paired t test p values for asym.

	100U vs. Mock	Virus vs. Mock	Virus+100U vs. Mock
CXCL9 4HPT	all values identical	all values identical	all values identical
CXCL9 12HPT	0.220	all values identical	0.374
CXCL10 4HPT	0.061	all values identical	0.078
CXCL10 12HPT	<0.001	all values identical	<0.001

Table 24: Paired t test p values for severe.

	100U vs. Mock	Virus vs. Mock	Virus+100U vs. Mock
CXCL9 4HPT	all values identical	all values identical	all values identical
CXCL9 12HPT	all values identical	all values identical	all values identical
CXCL10 4HPT	0.374	all values identical	0.374
CXCL10 12HPT	<0.001	all values identical	<0.001

Cellprofiler data analysis

Data is not log10 transformed.

Median of cellwise integrated intensity per donor per replicate is used as MFI.

Question 1: Determine if there are statistically significant differences between each time (30, 60, 120, 240 minutes) at each dose (0, 0.1, 1, 10, 100). Data represents fluorescence intensity in individual cells, collected across two independent experiments. Data is pooled across 10. For example, comparing 100U at 30 minutes to 100U at 60 minutes.

Table 25: Paired t test p values.

	60M vs. 30M vs. Prev. Time	120M vs. 60M vs. Prev. Time	240M vs. 120M vs. Prev. Time
0	0.056	0.165	0.002
0.1	0.016	0.447	0.033
1	0.005	<0.001	0.190
10	<0.001	<0.001	<0.001
100	0.002	<0.001	0.432

Question 2: Determine if there is a statistically significant difference between each dose (0.1, 1, 10, 100) to 0 at 30 minutes. For example, comparing 0 to 0.1, 0 to 1, 0 to 10, and 0 to 100 at 30 minutes.

Table 26: Paired t test

	0.1 vs. 0	1 vs. 0	10 vs. 0	100 vs. 0
30M	0.045	<0.001	<0.001	<0.001
60M	0.886	<0.001	<0.001	<0.001
120M	<0.001	<0.001	<0.001	<0.001
240M	0.669	<0.001	<0.001	<0.001

Question 3: Determine if there are any statistically significant differences between asymptomatic and severe at each dose (0, 0.1, 1, 10, 100) and each time (30, 60, 120, 240 minutes). For example, if there is a significant difference between asymptomatic 100U and severe 100U at 30 minutes

```
## boundary (singular) fit: see help('isSingular')
```

Table 27: Linear mixed models.

	30M	60M	120M	240M
0	-11.83 (CI=-19.47,-4.19, p=0.002)**W	-11.35 (CI=-18.47,-4.22, p=0.002)**W	-7.68 (CI=-15.05,-0.31, p=0.041)*W	-11.55 (CI=-18.78,-4.32, p=0.002)**
0.1	-9.76 (CI=-17.42,-2.11, p=0.012)*	-12.06 (CI=-19.85,-4.28, p=0.002)**	-8.52 (CI=-15.48,-1.56, p=0.016)*W	-10.86 (CI=-18.20,-3.52, p=0.004)**
1	-13.84 (CI=-25.47,-2.21, p=0.020)*	-14.17 (CI=-21.88,-6.46, p=<0.001)**	-15.38 (CI=-26.51,-4.25, p=0.007)**W	-17.29 (CI=-28.26,-6.32, p=0.002)**W
10	-32.03 (CI=-61.60, -2.46, p=0.034)*	-25.16 (CI=-41.36, -8.97, p=0.002)**	-36.29 (CI=-61.47,-11.12, p=0.005)**W	-38.88 (CI=-67.36,-10.39, p=0.007)**W
100	-35.82 (CI=-67.59, -4.06, p=0.027)*	-38.28 (CI=-71.35, -5.21, p=0.023)*W	-30.16 (CI=-54.91, -5.42, p=0.017)*	-53.35 (CI=-83.04,-23.66, p=<0.001)**

Table 28: Unpaired t tests.

	30M	60M	120M	240M
0	0.021	0.014	0.068	0.017
0.1	0.057	0.019	0.059	0.032
1	0.065	0.009	0.019	0.026
10	0.090	0.027	0.040	0.022
100	0.081	0.027	0.061	0.022

Appendix

This report was built with code from [this commit].