

Supporting Information

Banin *et al.* 10.1073/pnas.0808608105

SI Materials and Methods

Treatment Regimen. Treatment was initiated once the infected corneal lesion reached the threshold size detailed in *Materials and Methods*. Drops were administered q15 min for the first 4 h, q30 min between 4–12 h, q1 h between 12–48, and between 48–96 h q2 h during the day (16 h) with Tobramycin 0.3% ointment applied overnight. Treatment was ceased at 96 h, but animals were further followed until killed at different time intervals up to 14 d following treatment initiation.

Quantification of Disease Progression. To document the clinical progression of the infectious process, serial digital color photographs were taken: conventional color photographs were used to monitor the corneal infiltrate, degree of diffuse corneal opacity and scarring, level of hypopyon in the anterior chamber of the eye and extent of iris injection as described previously (1, 2). Photographs following application of fluorescein to the cornea were used to measure the area of corneal epithelial erosion.

Initial photographs were taken immediately following removal of the sutures and contact lens, and then again upon initiation of treatment. From this point on, conventional color photos were taken q2 h between 0–12 h, q4 h between 12–24 h, q6 h between 24–96 h and q24 h between 96 h and the time of sacrifice. Photographs of fluorescein staining were taken q4 h between 0–24 h, and from then on at the same time intervals in which regular color photos were taken. Degree of corneal opacity (outside of the main infiltrate), degree of iris injection, and severity of hypopyon formation were graded as previously described (1, 2). The areas of corneal infiltrate, scarring and erosion were measured from digitized photographs (using Photoshop 9.0 software) as shown in Fig. S1. Conversion from pixels to mm² and correction for photos taken at different distances from the eye was achieved by scaling according to the actual mid-corneal horizontal limbus-to-limbus diameter, which was measured using calipers in each eye. All gradings and measurements were performed by two observers masked to the treatment applied, and the average value was taken.

1. Banin E, *et al.* (2003) *Invest. Ophthalmol. Vis. Sci.* 44:2966–2972.

2. Morad Y, *et al.* (2005) *Invest Ophthalmol Vis Sci* 46:1640–1646.

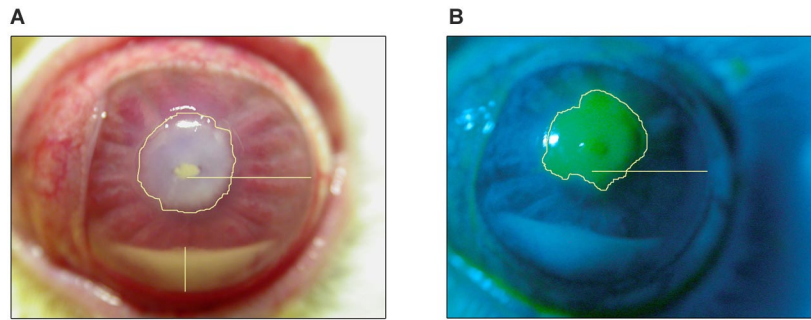


Fig. S1. Measuring and grading the infectious process from digital photographs. (A) Conventional color photographs were used to calculate the extent of corneal infiltrate and hypopyon level and to grade the degree of diffuse corneal opacity, corneal neovascularization, and iris injection. (B) Fluorescein photographs were used to calculate the area of epithelial defect. (Gm-treated eye, 96 h after treatment initiation; horizontal line is mid-corneal radius).

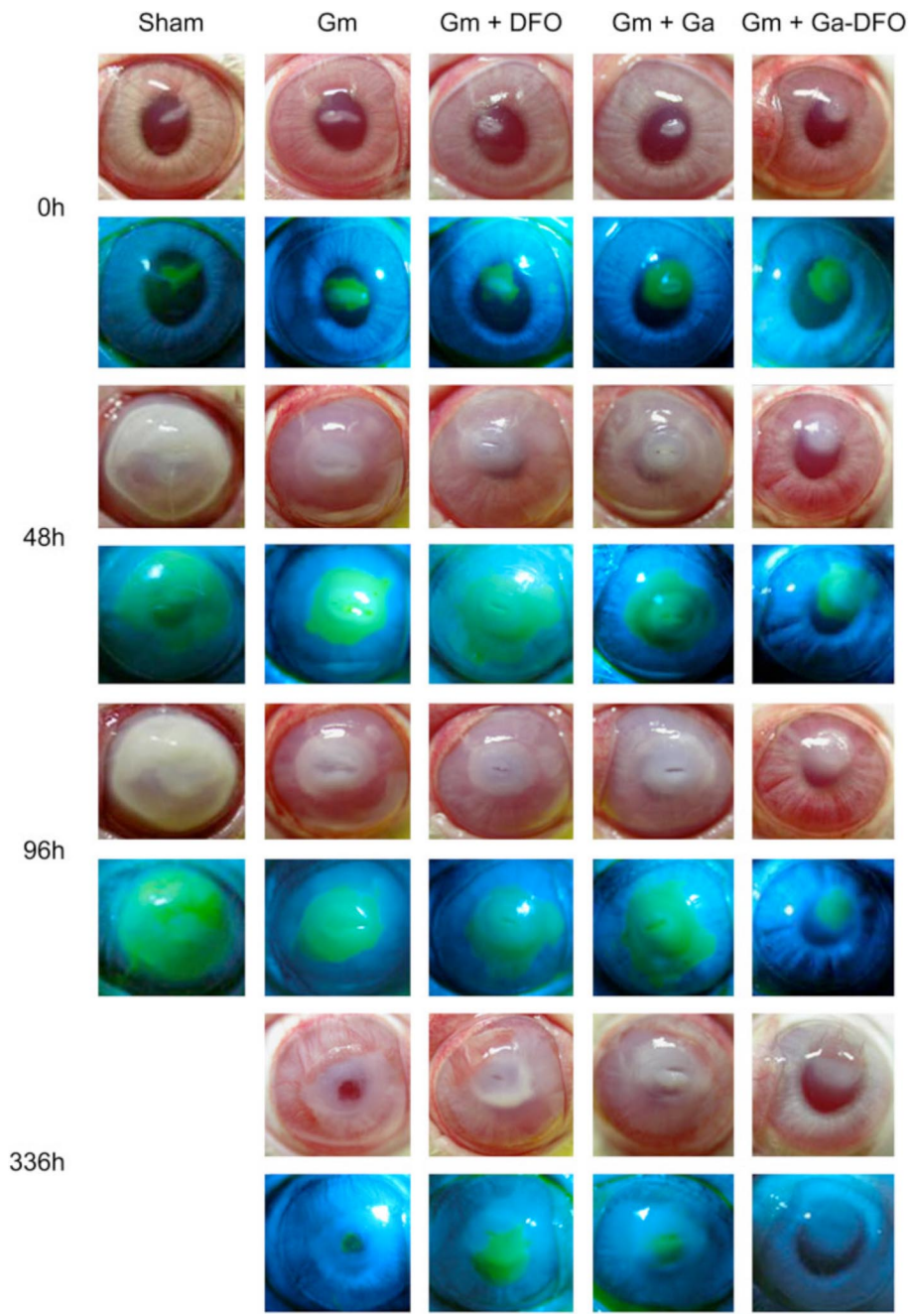


Fig. S2. Representative images of *P. aeruginosa* infected corneas in the different treatment groups over time. At each time point color photographs of infiltrate/scar and fluorescein staining of the corresponding epithelial defect are shown one above the other. The experiment in sham treated animals was terminated after 96 h due to the severe infection.

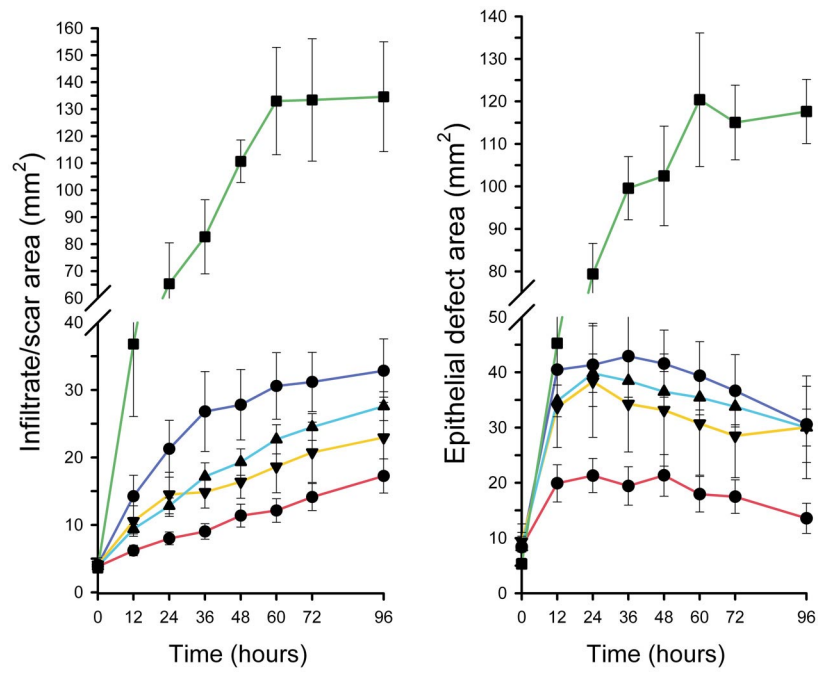


Fig. 53. Progression of keratitis in the different treatment groups. Extent and evolution of (*Left*) corneal infiltrate and (*Right*) epithelial defect over time in the different treatment groups over initial 96 h of treatment. Green squares, sham treated; blue circles, Gm+Ga; light blue triangles, Gm; yellow inverted triangles, Gm+DFO; red circles, Gm+DFO-Ga. Note the rapid and severe deterioration in sham-treated eyes as compared to all other groups. Eyes treated with Gm + DFO-Ga fared better than eyes treated with Gm alone at all time points. Each point represents mean area \pm SEM.

Table S1. Keratitis experimental groups and number of eyes examined

Treatment	Eyes examined, no.											
	Time after initiation of treatment											
	0 h	12 h	24 h	36 h	48 h	60 h	72 h	96 h	120 h	168 h	240 h	336 h
Gm	25	25	25	25	25	23	23	23	23	23	22	22
Gm + DFO-Ga	15	15	15	15	15	14	14	14	12	12	10	9
Gm + Ga	9	9	9	9	9	9	9	9	9	9	9	9
Gm + DFO	6	6	6	6	6	6	6	6	6	6	6	6
Sham	8	8	6	5	5	2	2	2	—	—	—	—