Deep learning identified an imaging biomarker of granuloma necrosis that is under distinct genetic control in experimental infection with *Mycobacterium tuberculosis*.

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OVERVIEW

Identifying who will develop tuberculosis remains a problem due to limited animal models and computational approaches. To address this problem, we use the Diversity Outbred (DO) mouse population. This population mimics human genetic diversity and lung granulomas when infected with *Mycobacterium tuberculosis* (*M.tb*). This led us to apply deep learning to identify highly susceptible DO mice from hematoxylin & eosin lung sections using only clinical outcomes (supersusceptible or not-supersusceptible) as class labels.

We used a Multiple Instance Learning (MIL) model previously determined to be 91.50 ± 4.68% accurate in diagnosing early mortality in *M.tb*-infected DO mice (i.e. those DO mice who succumbed to pulmonary tuberculosis within 60 days, classified as “supersusceptible”). Two board-certified veterinary pathologists determined the model identified an imaging biomarker corresponding to necrotic nuclear debris, providing insight into the neural network’s process. The imaging biomarker was quantified in lungs, and input for genetic mapping. This identified a genome location on chromosome 17 associated with granuloma necrosis. Inspection of the locus using the Mouse Genome Informatics database suggests genes in the Major Histocompatibility locus influence granuloma necrosis.

We use a multidisciplinary approach to understand tuberculosis, automatically detecting granuloma patterns for diagnosis and converting visual information for statistical analyses. We further show that pathologists’ interpretation provides insight into a “black box” process. These are major steps forward in trusting neural network’s diagnoses, and for high-throughput quantification of morphological tissue changes that cannot be measured by inspection or surrogate indicators.

METHODS

Aerosol infection of mice. Female Diversity Outbred (J:DO) and inbred founder strains were purchased from The Jackson Laboratory (Bar Harbor, ME) and infected with 25-100 *M.tb* bacilli using a nose-only exposure system.

Slide preparation and digital images. Mice were euthanized 60 days after infection; sooner if morbidity developed. Lungs were formalin-fixed, paraffin-embedded, sectioned, stained with hematoxylin and eosin, and digitally scanned.

Deep Learning Model. Multiple Instance Learning (MIL) model description, training, and validation has been submitted for publication elsewhere, and is available at github.com/cialab. Briefly, Phase 1 used feature extraction convolutional neural networks resulting in feature vectors for each instance in a bag. In Phase 2, each instance was pooled into an attention weight that was scaled such that their sum was 1 (i.e. softmax) and applied to their respective feature vectors from Phase 1.

Haplotype construction. Each DO mouse was genotyped using allele calls from GigaMUGA mouse genotyping array (Neogen, Lincoln, NB). A hidden Markov model (HMM) implemented in R software was then used to construct each DO mouse’s haplotype (i.e. genome).

Quantitative Trait Locus (QTL) genetic mapping. The proportion of *M.tb* infected lung tissue occupied by the MIL-identified imaging biomarker was input for genetic mapping. This is a statistical method that associates variation in a measured phenotypic trait with a specific location on the genome.

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RESULTS

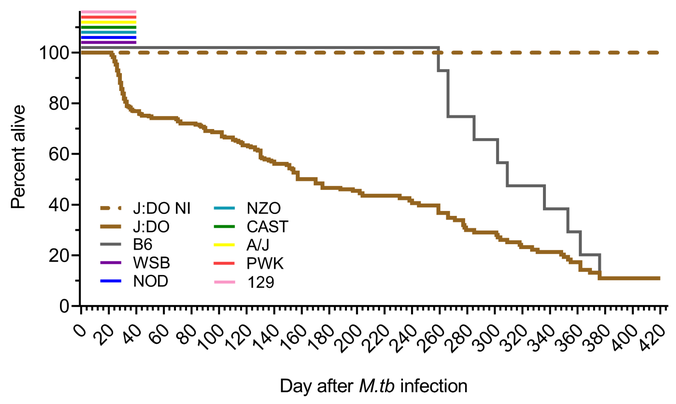
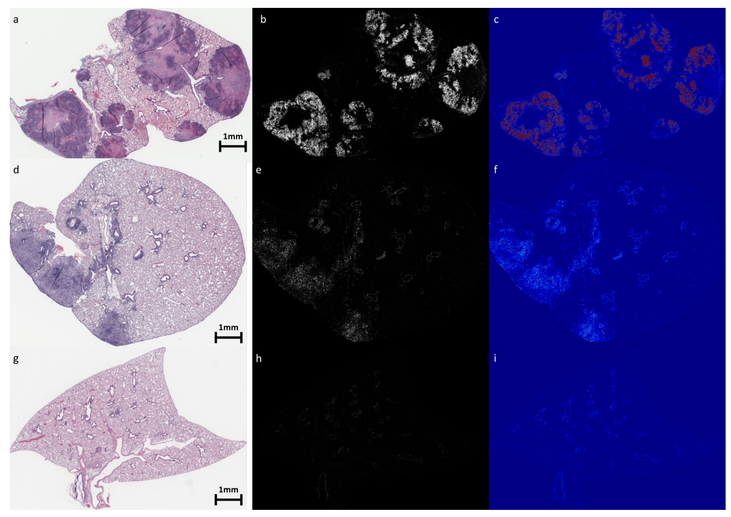


Figure 1 Genotype-dependent survival variability following *M.tb* infection of mice. Diversity Outbred (J:DO) mice (n = 654, brown solid line), and inbred mouse strains (n = 15 to 76, colored lines) were infected with ~25 *M.tb* bacilli by aerosol. Within 60 days of infection, nearly 30% of the DO population succumbed to pulmonary tuberculosis and were classified as supersusceptible. No other mouse strain or non-infected (NI) DO mice (n = 52, dashed line) showed mortality within the same period.

Figure 2 MIL identified regions of pyknotic nuclear debris in *M.tb* induced lung granulomas. H&E (left column) from a supersusceptible DO mouse that survived < 60 days (top left); A resistant DO mouse that survived > 60 days (middle left) and non-infected DO mouse (bottom left) are shown as representative examples. Brighter white (middle column) or more red (right column) regions correspond to areas indicative of supersusceptible granuloma features (i.e. imaging biomarkers). As expected, healthy, non-infected lung tissue in any class is not identified.



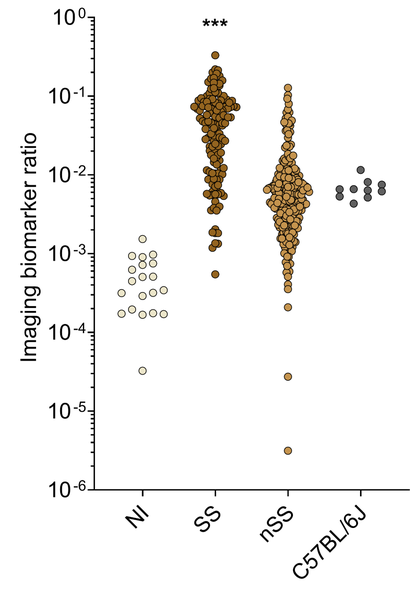


Figure 3 Quantification of granuloma necrosis imaging biomarker for statistical analysis. The imaging biomarker in Figure 2 was quantified as a ratio of necrotic/pyknotic regions to non-necrotic tissue and analyzed across multiple groups: Non-infected (NI) DO mice (n = 20) and *M.tb*-infected DO mice in supersusceptible (SS, n = 148) and not-supersusceptible (nSS, n = 266) classes. The biomarker was also quantified in the lungs of *M.tb*-infected C57BL/6J inbred mice (n = 10). The granulomas of supersusceptible (SS) DO mice contain significantly more of the imaging biomarker than all other classes. Each dot represents one individual mouse. Data was analyzed by ANOVA with Kruskal-Wallis post test (***p<0.001).

Figure 4 QTL mapping of granuloma necrosis using the MIL-extracted imaging biomarker identifies three peaks on chromosome (Chr) 17. The peak with the highest Logarithmic of the Odds (LOD) score on bottom panel is centered at 40 mega base pairs. This peak includes the mouse Major Histocompatibility locus, a region known to contain numerous immune response genes identified via the Mouse Genome Informatics database. The top panel with colored lines shows effects due to founder alleles. *Pwk* (red) and *nzo* (teal) alleles have strong opposite effects on granuloma necrosis.