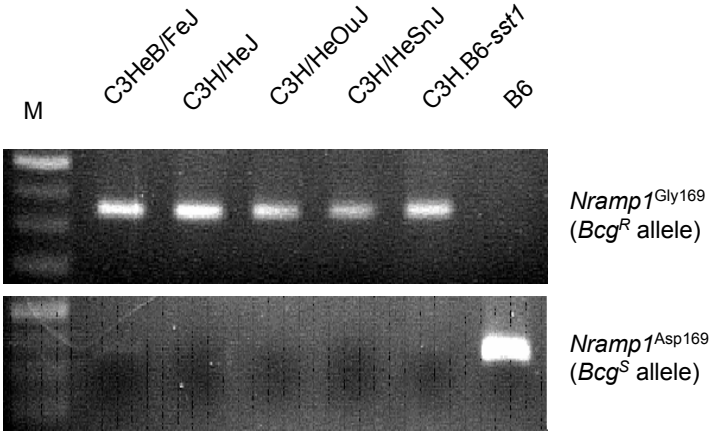
Alleles of the *Nramp1* gene in the mouse strains used in the study.



The RNA samples were prepared from the tuberculosis lung lesions of the C3HeB/FeJ, C3H/HeJ, C3H/HeOuJ, C3H/HeSnJ, C3H.B6-*sst1* and B6 mouse strains. Amplification of the *Slc11a1*(formerly known as *Nramp1*) cDNA was performed by RT – PCR using allele-specific primers that allow discrimination between the resistant ($Nramp1^{Gly169}$) and the susceptible ($Nramp1^{Asp169}$) alleles of the gene.

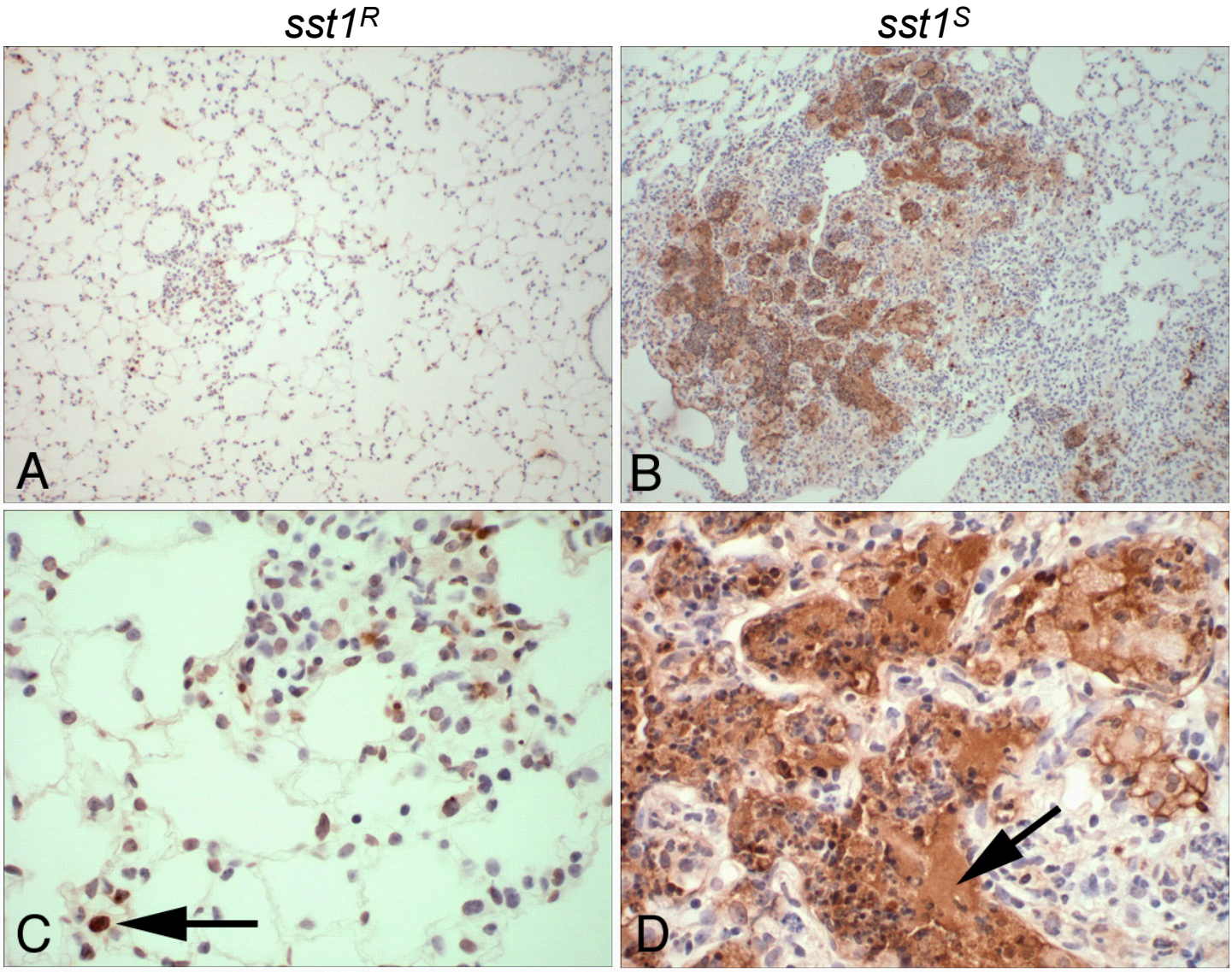
According to sequence analysis of *Nramp1* cDNA from inbred mouse strains (Malo D 1994; Medina E 1996), the BCG susceptibility is associated with a G to A substitution at nucleotide position 596 (Numbering begins at position 1 under GenBank accession No. L13732) which resulted in a Gly^{169} to Asp^{169} replacement within the conserved transmembrane domains (TM4) of the Nramp1 protein. Medina and colleagues (Medina E 1996 developed an elegant PCR assay for discrimination between the $Nramp1^{Gly169}$ (Bcg^R) resistant allele and $Nramp1^{Asp169}$ (Bcg^S) susceptible allele. Using the same set of primers and thermal cycling condition, we performed PCR on lung cDNA prepared from MTB infected mice. Gel electrophoresis of the PCR products clearly showed that all the C3H substrains used in our study and the C3H.B6-*sst1* ($sst1^R$) congenic strain expressed the $Nramp1^{Gly169}$ (Bcg^R) allele, while the B6 strain expressed the $Nramp1^{Asp169}$ (Bcg^S) allele. We also tested the genomic DNA isolated from mouse tails and confirmed the same allelic distribution.

Reference:

1. Malo D, Vogan K, Vidal S, Hu J, Cellier M, Schurr E, Fuks A, Bumstead N, Morgan K, Gros P. (1994) Haplotype mapping and sequence analysis of the mouse Nramp gene predict susceptibility to infection with intracellular parasites. *Genomics*. 23(1): 51-61

2. Medina E, Rogerson BJ, North RJ. (1996) The Nramp1 antimicrobial resistance gene segregates independently of resistance to virulent Mycobacterium tuberculosis. *Immunology*.88(4):479-481.

TUNEL staining of the tuberculosis lung lesions of the *sst1*^{R} and *sst1*^{S} congenic mice.



Apoptotic cells in the tuberculosis lung lesions of *sst1*^{R} (A and C) and *sst1*^{S} (B and D) congenic mouse strains were identified using a TUNEL assay and counterstained with hematoxylin.
In the *sst1*^{R} mice no necrosis is observed and some focal lesions include cells with TUNEL-positive apoptotic nuclei (C, arrow). In the tuberculosis lesions of the *sst1*^{S} mice the TUNEL-positive staining is associated with cytoplasm and extracellular exudative material (D, arrow), which is consistent with necrotic cell death and release of DNA fragments into the exudate.
The arrows show a TUNEL-positive nucleus in the resistant mice (C) and the TUNEL-positive extracellular exudative material in the susceptible mice (D). A and B - 100X; C and D – 400X original magnification.