Non small cell lung cancers (NSCLC) express cancer/testis antigens (CTA) genes and MAGE-A expression correlates with poor prognosis in squamous cell carcinomas. We addressed cytotoxic T lymphocytes (CTL) responses to HLA class I restricted CTA epitopes in TIL from NSCLC in an unselected group of 33 patients consecutively undergoing surgery. Expression of MAGE-A1, -A2, -A3, -A4, -A10, -A12 and NY-ESO-1 CTA genes was tested by quantitative RT-PCR. Monoclonal antibodies (MAb) recognizing MAGE-A and NY-ESO-1 CTA were used to detect CTA by immunohistochemistry. CD8(+) TIL obtained from tumors upon culture with anti CD3 and anti CD28 mAb and IL-2 were stimulated with autologous mature DC (mDC) and HLA-A*0101 restricted MAGE-A1(161-169) or MAGE-A3(168-176) peptides or HLA-A*0201 restricted MAGE-A4(230-239), MAGE-A10(254-262), NY-ESO-1(157-165) or multi-MAGE-A (YLEYRQVPV) peptides or a recombinant vaccinia virus (rVV) encoding MAGE-A and NY-ESO-1 HLA-A*0201 restricted epitopes and CD80 co-stimulatory molecule. Specificity was assessed by (51)Cr release and multimer staining. At least one CTA gene was expressed in tumors from 15/33 patients. In 10 specimens, at least 4 CTA genes were concomitantly expressed. These data were largely confirmed by immunohistochemistry. TIL were expanded from 26/33 specimens and CTA-specific CTL activity was detectable in 7/26 TIL. In 6, however, specific cytotoxicity was weak, (<40% lysis at a 50:1 E:T ratio) and multimer staining was undetectable. In one case, high (>60% lysis at 50:1 E:T ratio) MAGE-A10(254-262) specific, HLA-A*0201 restricted response was observed. Supportive evidence was provided by corresponding multimer staining. Although CTA genes are frequently expressed in NSCLC, detection of CTL reactivity against CTA epitopes in TIL from nonimmunized NSCLC patients represents a rare event.