demonstrated by IL-2 production on contact with immobilized or cellular CEA. In contrast to prior studies of isolated TCR chains that related increased tyrosine-based activation motifs in as a reason for superior signaling potency, these tests are the first to show that and are indistinguishable for T cell signaling when assayed in the context of the intact TCR complex. Further, Fab was equivalent to sFv as an IgTCR component for expression and antigen binding. When IgTCR was expressed on normal human T cells, cytotoxic potency was demonstrated at low E:T ratios, with T cell recycling and progressive tumor cell destruction. These studies establish a potentially important new immnotherapeutic modality for the treatment of CEA-expressing tumors.

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JAK Kinases and STAT Proteins in IL-12 Receptor Signaling

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IL-12 activate JAK2 and TYK2 and induce the phosphorylation of STAT4 and STAT3, but little is known how the activation of these signaling molecules is related to the biologic effects of IL-12. Using an IL-12-responsive T cell clone, We investigated their requirements for proliferation and IFN-g production of 2D6 cells. 2D6 lines maintained with IL-12 (2D6-12) or IL-2 (2D6-2) exhibited comparable levels of proliferation, but produced large or only small amounts of IFN-g, respectively, when restimulated with IL-12 after starvation of either cytokine. 2D6-12 induced TYK2 and STAT4 phosphorylation. But their phosphorylation was dramatically reduced in 2D6-2. The reduced STAT4 phosphorylation was due to a progressive decrease in the amount of STAT4 protein along with the passages in IL-2-containing medium. 2D6-12 and 2D6-2 similarly proliferating in response to IL-12 induced comparable levels of JAK2 activation and STAT5 phosphorylation. JAK2 was associated with STAT5, and IL-12-induced STAT5 phosphorylation was elicited in the absence of JAK3 activation. Above results also were confirmed in the Con A blast of B6 mouse spleen. These results indicate that TYK2 and JAK2 activation correlate with STAT4 phosphorylation/IFN-g induction and STAT5 phosphorylation/ cellular proliferation, respectively.

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Superinfection Exclusion of BVDV Occurs Not Only at the Level of Structural Protein-dispensable Viral Replication But Also at the Level of Structural Protein-required Viral Entry

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For a variety of viruses, the primary virus infection has been shown to prevent superinfection with a homologous secondary virus; however, the mechanism of exclusion has not been clearly understood. In this work, we demonstrated that BVDV-infected MDBK cells were protected from superinfection with a homologous superinfecting BVDV, one of the positive-sense RNA pestiviruses, but not with an unrelated rhabdovirus, such as vesicular stomatitis virus. Once superinfection exclusion was established by a primary infection with BVDV, the transfected infectious BVD viral RNA genome was shown to be competent for viral translation, but not viral replication. In addition, our results

also demonstrated that upon superinfection, the viral RNA genome of the viral particles was not transferred into the cytoplasm of BVDV-infected cells. Using newly developed system involving rapid generation of the MDBK cells expressing BVD viral proteins, we subsequently found that expression of the viral structural proteins was dispensable for a block occurring at the level of viral RNA replication, but required for a exclusion at the level of viral entry step. Furthermore, our data also showed that superinfection exclusion did not take place when BVDV-infected cells were passaged and become persistent. The failure of BVDV superinfection exclusion resulted from cellular alteration/adaptation of the persistently infected MDBK cells, but not from mutations in the BVD viral genome during passages. Altogether, these findings provide evidence that the superinfection exclusion of BVDV occurs not only at the level of viral replication in which the viral replicase are involved, but also at the level of viral entry with which the viral structural proteins are associated, and that a cellular factor(s) play an essential role in this process.

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HIV-1 Vaccine Development: Need for New Directions.

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The AIDS epidemic continues unabated in many part of the world. After near two decades, no vaccine is available to combat the spread of this deadly disease. Much of the HIV-1 vaccine effort during the past decade has focused on the viral envelope glycoprotein, largely because it is the only protein that can elicit neutralizing antibodies(Nabs). Eliciting broadly cross-reactive Nabs has been a primary goal. The intrinsic genetic diversity of the viral envelope, however, has been one of the major impediments in vaccine development. We have recently completed a comprehensive study examining whether it is possible to elicit broadly acting Nabs by immunizing monkeys with mixtures of envelope proteins from multiple HIV-1 isolates. We compared the humoral immune responses elicited by vaccination with either single or multiple envelope proteins and evaluated the importance of humoral and non-humoral immune response in protection against a challenge virus with a homologous or heterologous envelope protein. Our results show that (1) Nab is the correlate of sterilizing immunity, (2) Nabs against primary HIV-1 isolates can be elicited by the live vector-prime/protein boost approach, and (3) polyvalent envelope vaccines elicit broader Nab response than monovalent vaccines. Nonetheless, our findings clearly indicate that the increased breadth of Nab response is by and large limited to strains included in the vaccine mixture and does not extend to heterologous non-vaccine strains. Our study strongly demonstrates how difficult it may be to elicit broadly reactive Nabs using envelope proteins and sadly predicts a similar fate for many of the vaccine candidates currently being evaluated in clinical trials. We have started to evaluate other vaccine candidates (*e.g.* genetically modified envelope proteins) that might elicit broadly reactive Nabs. We are also exploring other vaccine strategies to elicit potent cytotoxic T lymphocyte responses. Preliminary results from some of these experiments will be discussed.

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Nrg1 is a Transcriptional Repressor for the Expression of Genes Involved in Glucose Metabolism in