Journal of Biomedical Science

J Biomed Sci 2000;7:169–172

Advances in Biomedical Research

The Sixth Annual Joint Scientific Symposium of NIH/FDA CAA and Washington DC Chapter of SCBA

The National Institute of Health (NIH)/US Food and Drug Administration (FDA) and Chinese American Association (CAA) and the Washington DC Chapter of Society of Chinese Bioscientists in America (SCBA) have succesfully sponsored five consecutive annual joint scientific symposia since 1994. Leading experts at the forefront of various biomedical fields have been invited to present their research findings. These joint symposia have sparkled spirited discussions and led to productive collaborations among Chinese bioscientists in the great Washington DC metropolitan area.

The sixth symposium was held in the NIH Bethesda campus (Bldg. 10, Lippsett Amphitheater) on October 9, 1999. This year's symposium highlighted recent advances in biomedical sciences, especially the HIV and tumor biology that has attracted attention from both the scientific community as well as the popular press. HIV infection has been a serious worldwide health concern. Prevention, intervention and treatment of this deadly diseae represent major challenges and opportunities to biomedical researchers. Three talks were devoted to this issue. The first two presentations by Drs. Sylvia Lee-Huang (NYU) and Hao Chia Chen (NIH) described the exciting discovery of new anti-HIV agents of promising clinical potentials. Several new anti-HIV agents including (1) MAP30 and GAP31 from medical plants and (2) AVL and AVR from urine of pregnant women and (3) RNase U and urinary lysozyme C from pregnant women were described. The third talk by Dr. Kuan-Teh Jeang (NIH) focused on the pivotal role of CXCR4 in the treatment of HIV infection.

The EGF signaling pathway plays an important role in tumorigenesis and angiogenesis. A new regulatory protein CAIR-1 that can control the releasing PLC-g in response to growth factor stimulation was presented by Dr. Howard Doong (NIH). Defects in the mismatch repair genes have been identified in various tumors. The human MYH, a homologue of *Escherichia coli* MutY, have been identified. Dr. A-lien Lu (University of Maryland) presented the linkage between inactivation of hMYH and tumor progression.

Immunological regulation and response to diseases via an intricate network of pathways in the human body: three presentations addressed this topic; Dr. XiaoDong Li (Johns Hopkins University) presented the rapid identification of differentially expressed genes in human TH2 cells and their functional significance in allergic asthma. Dr. J. Qian (American Red Cross) focused on the prevention and treatment of hemophilic inhibitors by exploiting the CD40L/CD40 and B7/CD28 pathways to develop an anti-hemophilic inhibitor in the murine model. Dr. Tiang-Li Wang (Johns Hopkins University) described the use of nucleic acid vaccine to prevent human papillomavirus-induced cervical cancer. Linkage E7 antigen with targeting signal of the MCH II pathway of LAMP-1 significantly enhances the potency of DNA vaccine.

Dr. Yufan Shi, Dr. Andrew Chang, Dr. T.-C. Wu, and Dr. Yunbo Shi provided valuable advice in organizing this symposium. We would like to thank Dr. M.K. Jeang, Cardiovascular Center, University of Texas of Health Sciences Center at Houston, the Science Division of Taipei Economic and Cultural Representative Office in US, and generous support from a number of companies. We especially thank Dr. K.-T. Jeang, a former president of NIH/FDA CAA, for his insights and enthusiastic and continuing support for this joint symposium.

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In Search of Novel Anti-HIV Agents

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For the past several years, we have been searching for novel antiviral and anti-tumor agents from nature products. From hundreds of samples investigated, we identified, purified to homogeneity, characterized and cloned a new class of anti-HIV agents with high potency and low toxicity from distinct and unrelated sources. The first group consists of anti-HIV proteins MAP30 (Momordica Anti-HIV Protein 30 kD) and GAP31 (Gelonium Anti-HIV Protein 31 kD) from medicinal plants and the second group consists of AVL (anti-viral lysozyme) and AVR (anti-viral RNase) from urine of pregnant women. MAP30 and GAP31 are isolated from medicinal plants *Momordica charantia* and *Gelonium multiflorum*, also known as bitter melon and Himalayan fruit, respectively. These compounds are unique in that they not only inhibit de novo infection by HIV-1 but also block the replication of the virus in already infected cells. We found that they affect HIV-1-infected cells with EC_{50}s (effective concentration at 50% inhibition) in the subnanomolar range (0.2–0.3 n*M*). They show no apparent cytotoxic or cytostatic effects on normal human cells even at 1,000-fold higher dose levels. MAP30 and GAP31 possess multiple therapeutic targets at different stages of the HIV-1 life cycle. We have characterized at least three biological activities that may be relevant to their therapeutic use. The first is an RNA N-glycosidase activity that cleaves the link between a ribose and adenine A4324 of 28S ribosomal rRNA. This inactivates the 60S ribosomal subunit and inhibits polypeptide chain elongation. The second is a DNA topological inactivation activity that renders HIV-LTR topologically inactive as substrates for DNA gyrase. This topoinactivation is similar to the effect of cellular topoisomerases in the presence of topoisomerase inhibitors. The third is inhibition of each of the three reactions catalyzed by HIV-1 integrase: 3′ processing of the viral DNA, strand transfer, and cleavage at the viral/target junction. It is thus important to define the extent to which each of these mechanisms contributes to desired antiviral and antitumor actions or to undesired cytotoxicity. We carried out structural and activity mapping of MAP30 and GAP31 by X-ray diffraction of crystals and by limited proteolysis. We identified and isolated proteolytic fragments of MAP30 and GAP31 that are fully active against HIV-1 but not in ribosome inactivation. These peptides are as active as their parent molecules in HIV-1 inhibition with EC_{50} in the range of 0.2–0.4 n*M*. They inhibit HIV-integrase activity and HIV-LTR topological interconversion, but they do not inhibit ribosome activity. These results demonstrate that the antiviral activity of MAP30 and GAP31 is independent from their ribosome-inactivating protein activity. This is of great significance and may provide useful insights in the design and development of antiviral and anti-tumor agents with specific therapeutic targets toward viral-infected and/or tumor cells, without cytotoxicity towards cellular targets. The second group of antiviral agents consists of AVL and AVR. To our knowledge, this is the first report that lysozymes and ribonucleases possess anti-HIV activity, and the first identification of these proteins as components present in crude β-core preparations that contribute to its anti-HIV effects. They represent a totally new class of therapeutic agents because they are naturally occurring human proteins that modulate viral infection. Details of these studies are presented in the following abstract.

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Mother Knows Best: From Pregnancy and the Discovery of Anti-HIV Proteins

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The transmission of HIV-1 from mother to fetus is rare during the first trimester of pregnancy when the secretion of hCG is high in the placenta. Consequently, hCG preparations were considered to have a role in the inhibition of HIV-1 transmission. Indeed, many hCG and its β-subunit (hCGβ) in particular were found to contain the anti-HIV activity both in vivo and in vitro studies. However, there has been controversy about whether some biological activities of hCGβ preparations are due to the β-subunit itself, or to other proteins present in the preparations. To determine whether proteins other than hCGβ itself might contribute to the anti-HIV activity of hCGβ preparations, we fractionated commercial preparations derived from the urine of pregnant women. We found that a significant portion of the anti-HIV-1 activity is associated with the β-core fraction. The β-core is a dimer of two peptide fragments of hCGβ linked by disulfide bridges. When a β-core fraction was purified by reverse-phase HPLC, the pure β-core molecules identified by N-terminal amino acid sequencing and SDS-PAGE were completely devoid of anti-HIV activity assayed by p24 production in chronically HIV-1 infected ACH2 lymphocytes and U1 monocytes. The bulk of anti-HIV activity was eluted behind the β-core fractions. Further purification of the fractions containing the anti-HIV activity by SDS-PAGE followed by Sephadex G-25 superfine, 18- and 18.5-kD fractions was identified by N-terminal amino acid sequencing as ribonuclease (RNase) U and 14 kD as urinary lysozyme C. Both purified enzymes exhibited not only respective authentic enzymatic activities but also anti-HIV activity. As expected, RNase U effectively degraded total RNA isolated from HIV-infected ACH2 lymphocytes. Similarly, ribonuclease A was found as 23 kD on SDS-PAGE in an extensively purified β-core preparation. We therefore designate the ribonuclease and lysozyme as anti-viral ribonucleases (AVR) and anti-viral lysozyme (AVL), respectively. Furthermore, commercially available lysozyme from chicken egg white, human milk, and human neutrophils and RNase A from bovine pancreas were demonstrated in these studies to possess activity against HIV-1. Since lysozyme is elevated in the urine of pregnant women and known to reduce the absorption of ectromelia virus, it may play important protective roles during pregnancy. This may explain why HIV infection from mother to fetus is rare. Collectively, these findings may offer new strategies for the treatment of HIV-1 infection.

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Molecular Insights toward Intervention against HIV-1

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Current interventions against HIV-1 infection have largely relied upon drugs that subvert the actions of viral reverse transcriptase and protease. Recent findings on the existence of cell surface coreceptors suggest that disruption of viral envelope/coreceptor interaction could potentially be an important strategy to prevent HIV-1 infection. We will discuss some insights and new findings on the interaction between T-tropic/syncytium-inducing HIV-1 and the CXCR4 coreceptor. We will propose an intervention strategy targeted toward this interaction.

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CAIR-1, PLC-Gamma and EGF-Signaling Pathway

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Phospholipase C-gamma (PLC-γ), a substrate for epidermal growth factor (EGF) receptor, is tyrosine phosphorylated upon stimulation. The activated PLC-γ hydrolyzes phosphatidylinositol 4,5-bisphosphate, generating inositol 1,4,5-trisphosphate and diacylglycerol, which subsequently elicit mobilization of intracellular calcium and activation of protein kinase C, respectively. The latter events have been reported to link to tumorigenicity, angiogenesis and metastasis. Our laboratory has reported a new regulatory mechanism for PLC-γ through the discovery of CAIR-1. CAIR-1 is a 74-kD cytoplasmic protein that is expressed in most mammalian tissues. CAIR-1 coimmunoprecipitates with latent PLC-γ in unstimulated human A2058 melanoma, MDA435 breast cancer and OVCAR-3 ovarian cancer cells. CAIR-1 does not pull down PLC-β, indicating that this binding is selective for PLC–. We have shown that upon EGF stimulation of MDA 435 cells, PLC-γ dissociates from CAIR-1. The dissociation of PLC-γ from CAIR-1 is log-linear to the EGF concentration administered ($r^2 = 0.99$, $EC_{50} = 55$ ng/ml). The release of PLC-γ from CAIR-1 nears completion 15–60 s with EGF stimulation (n = 3). The maximal tyrosine phosphorylation of PLC-γ occurs at or after 1 min, suggesting that the dissociation of PLC-γ from CAIR-1 may occur prior to PLC-γ translocation and tyrosine phosphorylation. We have shown that CAIR-1 is tyrosine phosphorylated upon EGF stimulation. However, unlike PLC-γ, CAIR-1 does not translocate to the cell membrane. We hypothesize that CAIR-1 is functioning as a cytosolic docking protein for PLC-γ and the tyrosine phosphorylation of CAIR-1 may be associated with the release of PLC-γ. We propose that proline-rich motifs in CAIR-1 may be the site of CAIR-1/PLC-γ binding. We have confirmed that CAIR-1 binds selectively with a glutathione-S-transferase (GST) construct containing the PLC– SH_3 domain but not to the GST-N-SH_2 or GST-C-SH_2. In summary, CAIR-1 provides a state of readiness for cells to respond to growth factor stimulation by releasing latent PLC-γ.

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Repair of Oxidatively Damaged Guanines in DNA and Carcinogenesis

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Escherichia coli MutY is an adenine DNA glycosylase active on DNA substrates containing A/G, A/C or A/8-oxoG mismatches and is also a weak guanine glycosylase on G/8-oxoG-containing DNA. 8-oxoG is the most stable and mutagenic product of oxidative damage to DNA, so MutY corrects the errors resulting from the replication of oxidatively damaged DNA. The active site of MutY contains Asp138 and Lys142 residues. The glycosylase activity is completely abolished in the D138N MutY mutant whereas the K142A mutant protein cannot form Schiff base intermediates with DNA substrates but has glycosylase activity. MutY has high binding affinity with 8-oxoG when mispaired with A, G, T, C, or inosine. The tight binding and fast catalytic activities towards A/8-oxoG-containing DNA are mainly contributed by the C-terminal domain of MutY. Alkylation interference experiments show that C-terminal domain of MutY makes direct contact with mismatched A and 8-onoG as well as several phosphate groups 5′ to the mismatched 8-oxoG. An *E. coli mutY* strain that produces an N-terminal 249-residue truncated MutY confers a mutator phenotype. These findings strongly suggest that the C-terminal domain of MutY determines the 8-oxoG specificity and is crucial for mutation avoidance by oxidative damage. We have identified the homologs of *E. coli* MutY (MYH) in yeast, mammalian nuclear and mitochondrial extracts. Human MYH can be found associated with the replication protein PCNA and the mismatch repair protein MSH2 and can be copurified with DNA polymerases in a DNA synthesome. The linkage of MYH repair to cancer is currently being pursued. Some malignant lung and breast cancer cell lines have much lower expression of hMYH than nonmalignant cells. Our hypothesis is that inactivation of hMYH will increase the frequency of G:C to T:A transversions, enhance hydrogen peroxide resistance and promote tumor progression.

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Identification of Differentially Expressed Genes in Human Th2 Cells

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Th2 cells are associated with allergic diseases, including atopic asthma, and are potential targets for developing novel therapies. The mechanisms governing the development of Th2 cell phenotype are

currently unclear, and the exact signaling pathways leading to Th2-dominant immune response remain to be determined. To this end, we have developed an efficient and reliable method to identify differentially expressed genes associated with the activation of Th2 cells. This was accomplished by combining cDNA enrichment procedure, suppressive subtractive hybridization (SSH) and high throughput cDNA array analysis. The use of SSH to equalize high and low copies of transcripts and to remove common sequences between tester and driver populations allows us to generate probes significantly enriched for differentially expressed genes. Allergen-stimulated human Th2 clones were used as the tester and either resting Th2 cells or stimulated Th1 cells as the driver populations. The genes differentially expressed in the tester but not in the driver can be examined directly by the use of cDNA microarray containing >45,000 cDNA clones from the IMAGE consortium. A database is being generated to contain lists of differentially expressed genes of Th2 cells at different time points after activation. In particular, those novel ESTs and genes localized on chromosomal regions showing linkage to asthma phenotype are being characterized for potential association with, and functional significance in, allergic asthma.

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Prevention and Treatment of Hemophilic Inhibitors by Targeting the B7s/CD28 and CD40L

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Hemophilia A is a hereditary bleeding disorder caused by factor VIII deficiency, and requires factor VIII infusion for replacement therapy. Gene therapy approaches to correct the deficiency are under clinical evaluation. However, treatment of hemophilia A patients with factor VIII is complicated by the frequent formation of inhibitory antibodies that inactivate factor VIII. We have used a murine model of hemophilia A to identify protocols for the prevention and treatment of anti-factor VIII antibodies. We have previously reported that the antibody response detected in hemophilic mice resembles that observed in inhibitor patients. Intravenous injection of factor VIII in these mice induces a T-cell-dependent anti-factor VIII antibody response that is completely blocked by the simultaneous administration of CTLA4-Ig. The nature of this effect was further evaluated in B7-deficient mice. While hemophilia A mice deficient in B7.1 developed high anti-factor VIII antibody titers, there was no anti-factor VIII antibody response when B7.2-deficient hemophilic mice were repeatedly injected with human factor VIII. Thus, B7.2-mediated costimulatory signals are essential for the induction of this antibody response. When anti-CD40L mAb was given to mice that had previously been primed to factor VIII, the anti-factor VIII titers were markedly diminished. The reduction in anti-factor FVIII titers observed in anti-CD40L-mAb-treated mice was significantly greater than the decay of anti-factor VIII antibodies in primed mice without anti-CD154 treatment. In addition, T cell responses to factor VIII were completely abolished by an anti-CD40L mAb treatment. Our data demonstrate that the maintenance of factor VIII inhibitors is dependent on the CD40L/CD40 pathway. Together, these studies suggest that strategies targeting the B7/CD28 and CD40/CD40L pathway are potential therapies to prevent and treat hemophilic inhibitors.

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Nucleic Acid Vaccines for Human Papillomavirus-Induced Cervical Cancer

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DNA vaccines represent an attractive approach for tumor immunotherapy because of their stability, safety and simplicity of delivery. In developing vaccines against human papillomavirus (HPV)-induced cervical dysplasia, we chose the E7 viral protein of HPV type 16 as a model antigen because it is associated with most cervical cancers. In addition, E7 is important in the induction and maintenance of cellular transformation, thus it is unlikely that tumor cell will downregulate the E7 protein. Recent advances demonstrated that T-helper responses play a critical role in initiating and maintaining immune responses. Therefore, we tested whether targeting E7 antigen to the processing pathway of MHC class II will enhance the presentation of E7 antigen to T-helper cells and thus, enhance the potency of DNA vaccines. E7 antigen was linked to the targeting signal of MHC II pathway of lysosome-associated membrane protein-1 (LAMP-1) and this chimeric DNA construct was designated as sig/E7/LAMP-1. DNA-sig/E7/LAMP-1 was injected into mice with a gene gun and the vaccine potency was tested with an in vivo E7-expressing tumor murine model (TC-1). In both tumor protection and tumor treatment assays, sig/E7/LAMP-1 DNA immunization elicited a more potent antitumor effect than E7 DNA. 100% of the mice were tumor-free after 2 g of DNA-sig/E7/LAMP-1 immunization. In contrast, with the same dose of DNA-E7 vaccination, all the mice grew tumors. Immune responses of immunized mice were determined by intracellular cytokine staining with flow cytometry analysis, cytotoxic T lymphocyte (CTL) assay, enzyme-linked immunospot (ELISPOT) assay, and ELISA assay. We detected a greater number of E7-specific T helper cells and stronger E7-specific antibody responses in mice immunized with the DNA-sig/E7/LAMP-1 than in mice immunized with DNA-E7 or no insert DNA. These results indicated an enhanced T-helper response generated by DNA-sig/E7/LAMP-1. Furthermore, DNA-sig/E7/LAMP-1 generated the highest E7-specific CTL activity and the greatest number of E7-specific CD8+ T cell precursors. In conclusion, our results indicated that linkage of the E7 antigen to the MHC class II pathway with endosomal/lysosomal targeting signal could greatly enhance the potency of DNA vaccines. Sig/E7/LAMP-1 DNA vaccines represent a promising approach for the control of HPV-associated malignancies and their precursors.