INTRODUCTION

NPC is a leading cause of death for large populations in southeast Asia. It is one of the 3 most common tumors in southern Chinese males and is not uncommon in northern Africa. It is a difficult cancer to diagnose, since it presents with a bewildering array of symptoms, and the nasopharynx is an area of the upper respiratory tract not readily visualized by direct examination. A consistent association of EBV with the undifferentiated type of NPC has been found, suggesting an etiological role for this virus. In addition, a number of environmental and genetic cofactors have been etiologically linked to NPC. Thus, studies aimed at NPC prevention and control must be multidisciplinary, involving many factors, including viruses, environmental chemicals, nutrition, and genetics.

The Fourth International Symposium on NPC addressed itself to current concepts and prospects in the epidemiology, early detection, monitoring, treatment, and prevention of the disease. Approximately 100 participants, representing 20 countries, attended. More than 55% of the participants were from Malaysia, Indonesia, Hong Kong, Singapore, the Philippines, Taiwan, the People's Republic of China, and India, all countries in which (with the exception of India) NPC is the one of the most frequent (incidence rate, 20 to 22 per 100,000 in males) tumors. The major participants, comprised of clinicians, pathologists, immunovirologists, and epidemiologists, focused on the following aspects: (a) clinical, including clinical presentation, pathology, and immunovirology; (b) etiology, including epidemiology, virology, cellular immunology, immunogenetics, chemical and biological carcinogens, and tumor promoters; and (c) prevention and treatment.

There were more than 50 presentations, including the current status reports of research in the laboratory, as well as of clinical and field investigations. The Symposium was opened with a historical summary by Prof. K. Shanmugaratnam (Singapore, Singapore Island), who emphasized that the distribution of NPC among the different Chinese dialect groups (Cantonese, Hokkien, Hakka, and Khek) in Singapore is the same as that of the corresponding groups residing in China. Moreover, he noted that the incidence of NPC does not change in high-incidence groups when they migrate to low-incidence regions, indicating the importance of genetic and/or cultural influences. He stated that association of EBV with NPC distinguishes it from all other human carcinomas and provides an opportunity to investigate how a ubiquitous virus might participate in the induction of a nonubiquitous tumor.

Clinical

The participants agreed that emphasis should be placed on early detection through screening procedures and by public education. Examination of the nasopharynx should stress visualization of the fossa of Rosenmüller, the area in which the initial onset of the malignant process most likely occurs. For early detection, all available techniques, such as radiographic scanning and EBV serology, should be utilized. The latter recommendation was based on the successful use of EBV serology for the detection of EBV antibodies in the sera of patients with occult primary tumors. It was also emphasized that adequate target biopsy materials should be provided from the fossa of Rosenmüller in cases in which diagnosis is uncertain or difficult. Above all, emphasis was placed on maintaining good communication between all medical disciplines.

Pathological Studies. The French, West German (Cologne), and WHO schemes of classification were compared, and it was recommended that a common classification system be established and adhered to for special investigations. However, it was considered necessary to circulate slides to identify problem areas and to determine whether the differences in histological types were due to biological or classification reasons. The group strongly recommended the creation of an International NPC Registry. This would provide a forum for establishing uniform comparisons of NPC between and within various ethnic groups, as well as for providing developing countries with a uniform system for diagnosis of NPC. It was also considered important to circulate histological slides from unusual cases (e.g., specimens in which all 3 histological types of NPC are found) so as to resolve differences in the classification of this cancer.

Special histopathological studies were considered important to define equivocal situations and to determine acceptable working standards. These include the definition of “dysplasia” of nasopharyngeal epithelium, “false-negative” and/or “false-positive” EBV serology, and the nature and functional composition of lymphoid stroma surrounding some undifferentiated NPC.

Prof. G. Klein (Institute for Tumor Biology, Stockholm, Sweden) mentioned that it is no longer sufficient to speculate about NPC cases having an association with EBV on the basis of serology alone; EBV DNA determinations in the tumor itself are mandatory. He also cautioned that in the EBNA test, activation of the C3 component can occur either by nonspecific means or by contamination with heterogeneous cell populations, which results in false-positive reactions. He recommended that more than one EBNA-positive and 5 EBNA-negative sera be run in parallel to test for EBNA-specific reactivity.

Clinical Immunovirology. Monoclonal antibodies were found
to be helpful in the dissection of antigen complexes. However, as yet, there is no monoclonal antibody against EBNA. A strong clinical-serological relationship was demonstrated between anti-body-dependent cell-mediated cytotoxicity titers and the status of the patient, the higher titers signifying a good prognosis and prolonged survival. IgA anti-viral capsid antigen was found to be more sensitive but less specific as a detection test than was IgA anti-early antigen. In situ, radioimmunoassay for EBV antigens was also found to be more sensitive than was the immunofluorescence test or enzyme-linked immunosorbent assay. Based on these findings, it was recommended that the new assays be assessed for specificity. Another test that was reported dealt with blocking of lymphocyte stimulation by EBV using sera from NPC patients with active disease. The blocking activity was specific for the IgA fraction of the serum, and no blocking activity was observed in sera from NPC patients in remission. One report stated that EBNA was also detected in paraffin sections; however, caution was advised based on loss of EBNA by fixation and embedding. Another detection assay reported was the single-stranded EBV DNA probe integrated into the replicative form of a phage carrier for hybridization. However, it was felt that the latter is not as yet feasible as a research tool. On the basis of the above, this group suggested continued evaluation of existing serological tests for specificity before defining the ultimate battery of tests to be used for the detection of NPC. Also, the tests should be performed in tandem with appropriate histopathological correlations and in sequential order to evaluate their prognostic significance. The group emphasized consideration of an International Reference Laboratory or Center for Reagents, Biopsy Materials, Technical Assistance, and Training, since the majority of the developing countries, particularly in the Far East and other parts of Asia (Indonesia and Malaysia are prime examples), lack well-characterized reagents and trained personnel.

Etiology

Epidemiology. There was a considerable amount of data presented on the sero-epidemiology of EBV in the People’s Republic of China, among the Eskimos of Greenland and Alaska, and in Malaysia. The group recommended further studies to identify high-risk groups in order to define the best candidates for intervention studies. Those found to have the preneoplastic condition, i.e., atypia or lymphoid hyperplasia, should be clinically monitored for early signs of neoplasia and as candidates for preventive approaches. The likelihood that a strong (i.e., highly penetrant) genetic element is involved in NPC is greater than for any other human carcinoma. The epidemiologists stressed the need for expanding genetic studies to include (a) first-degree relatives versus well-matched controls to determine whether such relatives are at increased risk for developing NPC, (b) the children of intermarriage between high- and low-risk individuals, and (c) multiple-case families. The lack of uniform genetic studies to date might be due to the fact that human cancer geneticists usually operate in parts of the world where NPC is not an important problem. The group felt it important to recruit human cancer geneticists to participate in every aspect of NPC, particularly in the design of well-controlled studies of NPC incidence and the factors involved.

There was also a total lack of cytogenetic information. Karyotyping was only reviewed in relation to some long-established cell lines. It was felt that the paucity of cytogenetic studies was due to the difficulty in obtaining cells in metaphase from solid NPC tumors. In view of the importance of cytogenetic studies in Burkitt’s lymphoma, banding studies in NPC were recommended. However, NPC patterns should not necessarily be expected to parallel those of Burkitt’s lymphoma.

Virology. Dr. E. D. Kieff (University of Chicago, Chicago, Ill.) started the session by presenting a comparison of mapped EBV genomes with those of other herpesviruses, showing that EBV was not as unique as had been thought. Furthermore, he indicated that the studies had focused on 3 gene regions that are highly transcribed in latently infected transformed cells. One region corresponds to a noncoding internal repeat that may have a regulatory function. A second corresponds to the IR3 repeat region. The size of the transcript varied in length between different cell lines, and a direct one-to-one correlation between this variation and the size of EBNA, which also varies between different lines, was observed. This region is of great interest in view of the postulated role of EBNA in transformation. Evidence was also presented for 2 different nuclear proteins in transformed cells. It was convincingly shown that an EBV DNA copy is integrated on a specific chromosome in EBV DNA-carrying B-cell lines, although most copies are episomal. The integration site is different in different tumors. In 2 NPCs, patterns obtained resembled the latentely infected Burkitt or lymphoid cells, and a third tumor showed a more lytic-type virus cycle, suggesting at least an abortive virus cycle. Two NPC-associated polypeptides were described (M, 44,000 and M, 22,000 proteins). However, it was not clear whether these are EBV- or NPC-associated markers.

The data on EBV infectibility indicated that the latent, EBV-positive, nonlytic interaction is still limited to the EBV-transformed B-cell lines and the NPC tumor cells. Some skepticism was expressed regarding EBV involvement in cell transformation, immortalization, and malignancy, unless in vitro transformation of epithelial cells can be demonstrated.

The review on the oncogenes and their significance in tumor biology stressed the need for oncogene studies in NPC. Two different ways were suggested. One involved probing for high transcription with some of the 17 known oncogenes, and the second involved transfecting NIH 3T3 cells with NPC DNA. It would be interesting and important to determine whether the oncogene sequences which emerged, if any, were viral or cellular in origin.

The virologists felt that particular efforts needed to be made to study the apparent tropism of EBV for nasopharyngeal epithelial cells and the infectious process. This is still not known, although a number of possible mechanisms were proposed during this session. The group also stressed the importance of establishing epithelial cell cultures from normal and NPC epithelial cells. Availability of such cells would enhance and stimulate more basic research.

Efforts should be made to utilize the advanced information derived in molecular and biological studies to acquire and/or produce pure reagents. The use of monoclonal antibody in the identification of various antigens in tumor or epithelial cells infected or transformed with viral or cellular DNA should be encouraged. The best and most available sources of materials for the above studies, i.e., the NPC biopsies or preneoplastic tissues from endemic areas such as the People’s Republic of China, Malaysia, Singapore, Hong Kong, or Indonesia, should be exploited for such investigations.

D. V. Ablashi et al.
CMI and Genetics. The session on CMI and genetics compared and discussed various CMI assays. The recommendations were for further comparison of various CMI assays to determine those most relevant for following the course of NPC. The group also noted the need for more extensive evaluation of cost-effective assays, such as the leukocyte adherence inhibition and indirect leukocyte migration inhibition, and suggested that the effector mechanisms relevant to immunity against EBV-infected cells and the use of EBV markers in the selection and classification of NPC patients for CMI be pursued.

With reference to HLA, the group from Singapore showed that Chinese NPC patients had lower frequencies of HLA A11 and A13 and higher frequencies of A2, AW33, B17, and BW46 than did matched controls within the same population. These investigators also demonstrated a lower frequency of DR4 in NPC patients than in controls (0.154 versus 0.270); however, the frequency of DR 'Blank' was higher. The frequency of 8WDRW6Y appeared to be higher among NPC patients of intermediate (1 to 5 years) and long-term survival (>5 years). Thus, its presence in newly diagnosed NPC patients is indicative of a good prognosis. Based on the above findings, the group recommended expanded application of genetic markers in endemic areas and in multiple-case families.

Other Cofactors (Carcinogens and Promoters). Considerable data was presented on chemical and biological carcinogens and tumor promoters, with particular emphasis on those found in southern China, where the incidence of NPC is very high. Thus far, it appears that the active carcinogenic elements identified in herbal medicine/drugs include diterpene esters based on tiglone, ingenome, and daphnane hydrocarbon skeletons, all of which, in crude form, are capable of inducing EBV early antigen expression in EBV nonproducer cells. Also, extracts derived from Croton tiglium, Euphorbia lathyris, Croton megalocarpus, and Jatropha curcas, compounds present in high-NPC areas, have been found to increase the transforming efficiency of EBV by 10-fold. Other promoters (lyngbyatoxin A, aplysiatoxin, and debromoaplysiatoxin) unrelated to the phorbol esters were found to induce the EBV cycle and bind to the same receptor as did the 12-O-tetradecanoylphorbol-13-acetate, perhaps triggering the event that leads to EBV induction. Similarly, the alkylating agent N-methyl-N-nitrosoquinuoline was found to enhance EBV transformation and antigen expression. Based on these findings, the group recommended closer review of the association of environmental carcinogens in affected geographical areas. It was also recommended that low-incidence areas adjacent to areas of high incidence be investigated in tandem to identify common and different compounds present in each environment. This would help determine which compounds were true carcinogens and/or promoters. Special attention should also be given to those areas in which specific candidate carcinogens are present in quantity for sustained periods over a wide territory. Attempts should be made to remove the carcinogen from one geographical portion of that territory and to monitor the population of the entire area for any changes in NPC incidence. This would only be feasible in situations in which exposure is due to custom or diet where the high-risk test population could be persuaded to desist in the use of such suspected carcinogens.

Prevention and Treatment

The last 2 sessions reviewed the findings on control of NPC. With regard to treatment, it was recommended that the clinical staging of NPC be unified by simultaneous recording of the HO, AJC, and UICC protocols, as is being done by the Mayo Clinic American NPC study group and by the University of Cologne. The radiotherapists stressed the need to develop optimal treatment protocols for primary and nodal NPC and to explore more effective and less toxic radiosensitizers and radioprotectors. In the area of chemotherapy, the need to develop reliable laboratory methods for optimally effective compounds was stressed. It was further suggested that adjuvant chemotherapy be considered for patients with extensive cervical node involvement. The exploration and development of effective protocols utilizing transfer factor and interferon for local and recurrent regional disease were encouraged. Such studies are feasible in endemic areas but must be done with standardized preparations. Even though data on the use of acyclovir and related compounds on herpes-virus infection are encouraging, the group thought that more research on their effect on EBV infections in humans is needed. Specifically, it is important to determine whether such compounds are effective in preventing EBV reactivation and which patients should be selected for such trials, e.g., those who have rising titers of EBV-early antigen (IgG, IgA) or EBV-viral capsid antigen (IgA) antibodies, or patients in remission.

The session on prevention reviewed the data on possible etiological factors, including EBV, genetic and racial susceptibility, chemicals (e.g., nitrosamines, carcinogenic hydrocarbons, benzoPyrene, and benzanthracene), compounds which act as promoters, life-styles or cultural patterns, socioeconomic status, geographic influences, and occupational exposures. The major focus on prevention was on the development and application of EBV subviral vaccines. The data on EBV membrane glycoproteins by Prof. M. Epstein (University of Bristol, Bristol, England) and Dr. G. Pearson (Mayo Clinic, Rochester, Minn.) supported the possibilities of developing a vaccine. However, during the discussion, there were dissenting opinions. The following recommendations came out of this session.

1. Identify and eliminate the major environmental risk factors associated with NPC. The association of specific chemicals with NPC should be reviewed, and efforts should be made to remove from the environment those found harmful. This would have to be approached on a regional basis.
2. Demonstrate protection against infection in laboratory animals using an EBV subunit vaccine.
3. Develop a pilot program, with emphasis on technology for large-scale production of a subunit vaccine.
4. Perform safety testing of a subunit vaccine in seropositive volunteers. Some participants suggested test trials in seronegative volunteers at risk for infectious mononucleosis and/or in NPC patients at risk for recurrence of disease.

The informal discussions during the active and more informal evening sessions all supported the establishment of International Working Groups for NPC Research, Clinical Application, and Epidemiology. Coordination among groups and disciplines should be encouraged and achieved through a common body willing to undertake this responsibility.

The participants further felt the need of more informal exchanges of ideas, materials, and investigators, the latter particularly from the developing nations in which NPC is a significant health problem. They particularly stressed collaboration with investigators from the People's Republic of China, since NPC ranks as the No. 1 tumor in southern Chinese (78.23%), according to data provided by the Tumor Hospital Zhongshan Medical
College for the years 1964 to 1981. Such collaborations, again, could be arranged through a common body, e.g., the National Cancer Institute or WHO.

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Fourth International Symposium on Nasopharyngeal Carcinoma Application of Field and Laboratory Studies to the Control of NPC


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