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Meeting Report

International Symposium on Nasopharyngeal Carcinoma—Basic Research as Applied to Diagnosis and Treatment

The XII International Symposium, sponsored by the Society for the Cancer Campaign of Northrhine-Westfalia (Gesellschaft zur Bekämpfung der Krebskrankheiten Nordrhein-Westfalen E.V.), was organized by Professor E. Grundmann, President of the Society for the Cancer Campaign of Northrhine-Westfalia; Professor G. Krueger, University of Köln; and Dr. D. Ablashi, National Cancer Institute. Approximately 65 investigators, representing all aspects of NPC[1] research and treatment, from the United States, Colombia, Denmark, Japan, West Germany, England, France, Italy, Greece, Tunisia, Hong Kong, Singapore, People's Republic of China, Malaysia, and the Netherlands, participated. The symposium was held in the Haus der Wissenschaften in Düsseldorf, Federal Republic of Germany, from October 23 to 25, 1980. This was the third international symposium on NPC and, like the preceding two, was multidisciplinary in nature. The emphasis of this meeting was on progress, since there had been three years between the third and second which had been held in Kyoto, Japan, in 1977, and to determine the areas of activity for future efforts.

NPC is not a common tumor among Caucasians but is the most prevalent ear, nose, and throat tumor in male Cantonese Chinese in Southeast Asia and the fourth most common tumor in Northern Africa. Recent epidemiological and clinical data indicate that NPC is also prevalent in young age groups in Tunisia, Algeria, Morocco, South America (Colombia), and to a lesser extent, among blacks in the United States. This tumor, which develops in the postnasal space, represents a unique model for studying the contributions of genetics, viruses, and chemicals to the etiology of at least one prevalent human cancer. It has long been established that the development of NPC is multifactorial and that its pathogenesis proceeds in multisteps. The etiological role of a lymphotropic herpesvirus EBV has been demonstrated by EBV DNA hybridization, the presence of EBNA in the carcinoma (epithelial) cells, and by serology.

The reviewers of the pathology of NPC supported the present WHO classification even though it was not fully satisfactory to all. The modifications proposed by the German and French groups represent valuable additional viewpoints which will be further tested. The pathologists all agreed that more tissues and more than 2 biopsies were needed before further review of the histological classifications could be undertaken. Also included for further study were tumors of the parotid gland and those with secondary involvement of the nasopharynx. They recommended that tumor imprints be examined for EBNA and that histological sections be circulated among contributing pathologists. It was agreed that more than 100 NPC cases would be needed for reclassification according to the WHO, French, and German systems.

The clinical staging of NPC was reviewed. Discussions covered clinical status in relation to EBV serology and survival data of NPC cases in the United States and Hong Kong. It was recommended that the participants continue to use the current staging criteria and compare their findings with Dr. Ho's staging and serology, since his staging always correlated well with serological findings in Chinese patients according to Drs. W. Henle and G. Henle, who performed the serological tests on coded sera sent to them by Dr. Ho; *i.e.*, elevation of EBV antibodies were generally associated with increase in stage. It is important and desirable to record the size of individual tumors in the neck but not in the nasopharynx since it is difficult to measure the primary in the nasopharynx. The use of EBV serology as suggestive indicator load was strongly encouraged in view of its apparent validity in reflecting changes in the neoplastic state.

The virological data on NPC revealed a good correlation between EBV antibodies and undifferentiated or poorly differentiated NPC. These data confirmed previous reports which suggested that NPC of the well-differentiated type may not be EBV related at all. The group recommended serological study of more well-differentiated NPC to determine whether this histological type should be dropped from consideration in terms of an EBV etiology.

The molecular data showed no major variations between EBV strains. Moreover, the EBV genome was found in the parotid glands of healthy persons, suggesting that these glands might be the reservoir of EBV replication after persistent infection. Evidence of EBV DNA was also found in the salivary glands of Cantonese Chinese from the People's Republic of China and Alaskan Eskimos. The virologists recommended that these glands be included in tissues studied for evidence of EBV infection.

The question of how EBV was associated with epithelial cells was raised. While the opinion was expressed that the metaplastic change from columnar epithelial cell to the squamous one was a prerequisite, it was suggested that the transfer of EBV could be brought about by close contact of lymphocytes with epithelial cells within the confines of the Waldeyer ring. The EBV in lymphocyte-positive cells could conceivably induce fusion with epithelial cells. The latter could then become carriers of the EBV genome, perhaps potentiating their growth and transformation under circumstances favorable to such an event. This could be explored in terms of identifying possible growth factors conducive to support of epithelial cells *in vitro* for virological and fusion studies. An expanded effort to isolate EBV from NPC was also recommended.

The group reviewed the feasibility of an EBV vaccine for initial trials perhaps in EBV antibody-free children 10 to 15 years old to prevent mononucleosis and in infants in Africa

[1] The abbreviations used are: NPC, nasopharyngeal carcinoma; EBV, Epstein-Barr virus; EBNA, Epstein-Barr virus nuclear antigen; VCA, viral capsid antigen; ADCC, antibody-dependent cell-mediated cytotoxicity; ACIE, Anticomplement immunoenzyme CMI, cell-mediated immunity.

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after their maternal antibody protection had dropped. EBV membrane glycoproteins as a source of vaccine were recommended in the above populations, as well as in NPC patients in remission, to prevent recurrence of tumor. Trial vaccines, if feasible, would have to be tested in suitable animal model systems prior to use in humans.

EBV serology in relation to NPC was presented and reviewed. The populations studied included NPC cases and controls from the United States, West Germany, Algeria, Malaysia, Tunisia, People's Republic of China, Greenland, Hong Kong, Singapore, and Greece. The most prevalent EBV-associated antibodies were IgA, VCA and IgG early antigen antibodies. Wider use of these antibodies in NPC cases, family controls, and the general population was encouraged, since IgA VCA antibody had been found very useful in diagnosis and identification of high-risk individuals. The IgG early antigen antibody was found to be of considerable prognostic value. ADCC was of probable prognostic value, since higher titers in the majority of patients studied correlated well with good survival and response to therapy and low titers with poor survival, recurrence of tumor, and poor response to therapy. These antibodies would appear to be useful markers. The evidence that IgA antibody was capable of blocking ADCC suggested that γ-globulins should be explored in terms of immunotherapy by way of altering the immune status of patients found to be ADCC deficient.

The group also recommended that epidemiological studies in high-, intermediate-, and low-risk NPC populations be continued. However, larger numbers should be included and each case better defined.

The data in HLA type and risk for NPC, derived from 313 Chinese patients and 330 normal controls, were presented. HLA Aw19/B17 was associated with higher risk and A11 with decreased risk. NPC in HLA type A_2BW46 was seen in the ≥30-year age group. The B13 type was seen in older onset patients. In newly diagnosed cases, B17 showed a poorer survival rate (<5 years) and A_2B13 a higher survival (>5 years). Moreover, the data associated B17 with poor survival in comparison to B13 where survival was quite good. These data were reviewed in depth by the group. The participants felt that studies on the association of HLA types and NPC should be continued, in combination with follow up of normal controls of the same HLA profile. They suggested that parallel studies be continued in NPC high-risk populations other than the Chinese described above. Moreover, further collection of data on DR type is desirable so that NPC risk as related to these antigens can be determined. Studies should be expanded to characterize the *IR* locus. This may provide useful information particularly in regard to suppressor cell activity.

The IgA VCA antibody study in 310 immediate blood-linked relatives and 99 patients from Hong Kong demonstrated IgA antibody titer <5 in 3% of individuals ≥35 years of age and only in 2% of people <35 years of age. One individual with a titer of 40 was found to have an asymptomatic carcinoma confirmed by biopsy in his nasopharynx; those with titers of 5 to 10 were negative for tumors. These observations supported previous findings of the Hong Kong group wherein they demonstrated the association of elevated IgA antibody with the development of NPC. The data presented from the Mayo Clinic group on 40 patients with occult tumors in the head and neck suggested that IgA VCA antibody was an excellent aid in diagnosis of NPC.

In a report from the People's Republic of China covering a 16-month period, 62 NPC cases were identified with the use of IgA antibody. In these studies, the investigators also used immunoenzymatic (ACIE) methods in place of EBNA which could not be practically applied in certain areas of China. In these studies, ACIE proved more specific and sensitive for early detection of NPC, especially when used in combination with other antibodies to EBV. The ACIE and EBNA tests further suggested that the EBV genome was present in normal ciliated columna epithelial cells and hyperplastic cells of the nasopharynx. It would thus appear the presence of EBV DNA might be related to the initiation of the malignant process in combination with other contributing etiological factors. It is suggested that the ACIE test be tried by other groups to determine its usefulness and repeatability for routine screening of population at risk. Thus, in view of the evidence presented, the use of IgA antibody in detection of NPC or identification of individuals at risk was of demonstrated value and should be continued.

The interaction of virus with promoters commonly found in the areas of Southeast Asia where NPC is prevalent supports the importance of cofactors in the etiology of NPC. The *Fusobacterium nucleatum* which is part of the normal microbial flora of the mouth and nasopharynx in humans produces *n*-butyric acid in culture media. The media proved to be effective in EBV induction. Moreover, croton roots from a leafy shrub of Euphorbiacea family, *Croton tiglium*, and also other plant species such as *Euphorbia lathyris* are widely used herbal drugs in the areas where NPC is prevalent. Data was also presented which showed that, when the bacterial inducer and the plant promoters are used in combination, their EBV-inducing effect is markedly enhanced. It is the general feeling that exploration of such interactions should be continued. The group specifically recommended that virologists and clinical carcinogenesists be encouraged to participate in these studies.

CMI in NPC patients was discussed in terms of skin testing, T- and B-cell values, and comparison of the leukocyte migration inhibition factor test and lymphocyte transformation assays. It was pointed out that some of these studies have been hampered by problems in preparing and standardizing the antigens used in the CMI assays and skin testing. The T- and B-cell data suggested that IgA-producing cells were significantly elevated in patients with undifferentiated carcinoma as opposed to IgG predominance in squamous cell carcinomas. Also, local T-cell levels correlated with elevated local IgA-producing cells, a finding which could be of prognostic value in following NPC cases. Data were also presented on proper preparation of standard EBV antigens using infected and uninfected cells for use in the CMI assay. Antigens could be fixed in a formalin base and stored in the refrigerator over long periods. Materials handled in this way were tested by leukocyte migration inhibition factor and lymphocyte transformation assays in NPC patients and proved reliable and effective. The participants felt it was important to continue studies on the use of CMI surveys in the evaluation and management of NPC patients and encouraged the use of the standardized antigen preparations by all collaborating groups to obtain comparable data.

The data on kidney transplants and other immunodepressed patients was discussed. It was observed that such patients do not respond serologically in the usual way. There also appears to be higher frequency of lymphoma incidence in immunodeficient populations. Expansion of studies in such patients is

clearly needed to gain a better understanding of the interrelationship of serology, pathology, clinical picture, EBV association, host genetics, and host immune surveillance.

Data presented on EBV hybridization experiments indicated that the p32-labeled DNA probe representing the repeated fragments of EBV genome used in the Southern blot method for detection of EBV DNA in NPC tissues was sensitive and required small amounts of DNA. This assay was enthusiastically recommended for future studies.

The last aspect of the symposium dealt with trends in NPC treatment. Although interferon had been demonstrated to have some therapeutic activity in NPC patients, small case numbers did not allow final conclusions. It was suggested a larger controlled study be initiated using interferon on selected patient material which should be pretested immunologically and virologically by standardized methods. The group also expressed the hope that it would be possible to get larger quantities of purified interferon for use in NPC. They also had questions regarding which type(s) of interferon was most effective. Radiotherapy is still the treatment of choice, but other treatment modalities were important when patients did not respond. Trials of multiagent chemotherapy [bleomycin-methotrexate-Velban-1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea] in NPC patients with advanced disease (T4N3b) were encouraging; many of the patients responded favorably, and the median survival period was increased. Based on these findings, the group recommended that new patients be treated routinely with adjuvants in addition to radiotherapy; *i.e.*, radiotherapy followed by chemotherapy and the use of multiagent therapy in recurrent disease. Transfer factor, still in the laboratory stage, was another approach to eventually consider. Cryosurgery is sometimes useful in controlling the primary. The group also discussed the possible application of immunotherapy (*i.e.*, administration of high ADCC γ-globulins to low ADCC patients), the intervention by vaccines during remission, and the use of antiviral drugs, such as Acyclovir. They felt that all of the above approaches should be followed closely and perhaps tested in selected cases.

Lastly, it was emphasized that NPC was a unique tumor which would require collaboration between basic and clinical investigators at the national and international levels and that such collaborations should in every way be encouraged.

The proceedings of the symposium will be edited by E. Grundmann, G. Krueger, and D. Ablashi and will be published in 1981 as Cancer Campaign, Vol. 5, by Gustav Fischer Verlag, Stuttgart/New York. Further information on the availability of this publication can be obtained from Professor E. Grundmann.

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