Influenza-Virus-Induced Hyperplasia of the Respiratory Tract of the Hamster

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ABSTRACT

Hamster-adapted A/PR/8 influenza virus infection followed by a secondary insult consisting of 0.25 ml of 0.5% gelatin-saline instilled directly into the trachea causes epithelial hyperplasia of both the trachea and lung. The extent of the response is related to, and dependent upon, the time interval between virus infection and gelatin-saline administration; the optimal time interval is between 12 and 18 hr. The presence of hyperplasia was measured by ³H-thymidine incorporation. Hyperplasia of the trachea is first apparent 3 days after virus infection, peaks at the fourth day, and is no longer present by the twentieth day. Hyperplasia in the lung begins 4 days after virus infection, peaks at the sixth day, and is undetectable by the twentieth day. Additional studies are in progress to determine the effect of virus-induced hyperplasia on benzo(a)pyrene-ferric oxide initiated respiratory carcinogenesis.

Introduction

Previous work at IITRI has demonstrated that intranasal administration of hamster-adapted A/PR/8 influenza virus, followed at selected intervals by an additional intratracheal insult, produces a hyperplastic bronchial and bronchiolar epithelium. The hyperplasia occurs whether the additional insult is nickel oxide, gelatin-saline, or saline, and appears to be related to the time interval between infectious challenge and administration of the insult. Markedly hyperplastic bronchial and bronchiolar epithelia, adenomatosis, pleural thickening, and an intense interstitial response were present in the lungs of hamsters upon histological examination.

The studies reported here were designed to determine the time relationship between virus infection and a secondary tracheal insult on the development of epithelial hyperplasia, which may be a facet in the expression of respiratory cancer. The objective is to test whether a maximum hyperplastic response from the virus infection with a subsequent administration of a carcinogen at the proper time leads to a high incidence of tumor. Epithelial hyperplasia can be induced by

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a number of conditions, including vitamin A-deficiency, exposure to injurious chemicals, and virus infection. There seems to be some relationship between viral infection and carcinogens. This relationship is either direct as a cofactor, or indirect as an increase in susceptibility. Studies documenting this relationship have been published (KOTIN, 1958, 1963; STRAUB, 1937). We hope to obtain additional data, utilizing the hamster, and to build on this work. Such studies will provide an animal model to investigate the concept that epithelial proliferation may lead to an increased susceptibility to chemical carcinogenesis.

Materials and Methods

Random-bred male Syrian golden hamsters were housed in groups of 5 in polystyrene cages with a filter bonnet (Filtex, Appleton, Wisconsin). The animals were fed a pelleted stock diet (Rockland Mouse/Rat Diet, Glen Ellyn, Illinois) and given bottled tap water ad libitum. Cages, bedding, filter bonnets, and water were steam-sterilized and changed twice a week. Temperature in the room was maintained at 24°C with a 12-hour light-dark lighting cycle.

Influenza virus, A/PR/8 strain, was adapted to hamsters by serial passaging, with a final 20% lung suspension used for infectious challenge. The virus was identified by use of specific A/PR/8 influenza virus antiserum obtained from the National Institutes of Health.

Hamsters were anesthetized with dry ice (CO₂) and 0.1 ml of the 20% lung suspension was placed on the septa between the nares. The viral suspension was carried into the lungs upon inhalation. A single 0.2 ml dose of gelatin-saline (0.5% gelatin) was injected intratracheally, as a secondary insult, according to the method of SAFFIOTTI (1968), at various time intervals after infectious challenge. Each animal received an intraperitoneal injection of 300 μCi-tritiated thymidine 1 hour before sacrifice. The lungs and trachea were removed en block from each animal, weighed by sections, frozen, and examined for tritiated thymidine incorporation into DNA, and by autoradiography for thymidine labeling of epithelial cells. Both trachea and lung samples were analyzed for thymidine incorporation into DNA. Results were expressed as DPM/μg DNA.

 Autoradiography of tracheal sections from animals injected with tritiated thymidine was carried out to confirm the biochemical data. Cross-sections of trachea were fixed in s-collidine-buffered 5% glutaraldehyde, embedded in Epon 812 containing 5% Araldite, and sectioned with an ultramicrotome. Photomicrographs of 1 μm tracheal sections were taken at a microscopic magnification of 32.5 X. The negatives were enlarged 8.25 X to a total magnification of 268 X. The epithelial length was measured at the junction of the epithelium and connective tissue from the photographic prints, with correction for microscopic and photographic enlargement. Labeled cells were microscopically counted in the same area on the original tracheal section through a 100 X oil-immersion objective lens. The results were then expressed as mean number of labeled cells per 10,000 μm of epithelial length ± standard error (labeling index).