The Reversal of Keratinized Squamous Metaplastic Lesions of Vitamin A Deficiency in Tracheobronchial Epithelium by Vitamin A and Vitamin A Analogs in Organ Culture: A Model System for Anti-Carcinogenesis Studies

Michael B. Sporn, Gerald H. Clamon, Joseph M. Smith, Nancy M. Dunlop, Dianne L. Newton, and Umberto Saffiotti
Lung Cancer Branch, Carcinogenesis Program, National Cancer Institute, Bethesda, MD 20014, USA

ABSTRACT

Normal cellular differentiation in tracheobronchial epithelium is dependent on vitamin A. In the absence of vitamin A the normal ciliated and mucus-producing cells of this epithelium are replaced by squamous cells, which are neither ciliated nor mucus-producing, but which do produce keratin. We have developed an in vitro system for studying the above process, using organ culture of hamster tracheas in chemically defined, serum-free medium. Keratinized squamous lesions of tracheobronchial epithelium can be induced by absence of vitamin A in the defined medium. Addition of vitamin A or its analogs to the culture (after such lesions have formed) induces reversal of keratinization and growth of a new ciliated and mucus-producing epithelium. A single one-day dose of all-trans-retinyl acetate or all-trans-retinoic acid is sufficient to effect reversal of keratinization. The hamster tracheobronchial organ culture system is also being used in our laboratory to determine whether vitamin A and its analogs are capable of reversing squamous metaplastic or other preneoplastic lesions that are induced by chemical carcinogens.

Introduction

This paper presents the results of some of our first attempts to develop a model system to study the process of anti-carcinogenesis in respiratory epithelium in organ culture. Two very basic clinical facts about lung cancer in man encourage us to proceed with this approach. The first basic fact is that the development of bronchogenic squamous cell carcinoma is a multistage process, with an extremely long latent period in man (AUBERBACH et al., 1961, 1962a; SELIKOFF et al., 1967; SACCAMANO et al., 1971); the latent period for development of clinically manifest disease may be 20 years or more from onset of exposure to the carcinogenic insult. Although many different sets of nomenclature have been used, it is generally accepted that during development of bronchogenic squamous cell carcinoma, the respiratory epithelium progresses through a series of stages of metaplasia, with increasing frequency of atypical metaplastic cells, before development of invasive carcinoma. The same process can also be shown to occur during respiratory carcinogenesis in the experimental animal (SAFFIOTTI et al., 1968; HARRIS et al., 1972; SCHREIBER et al., 1974). The second basic fact is that the progression from one stage of metaplasia to the next is not inevitable. Studies with ex-smokers tell us that respiratory epithelium has a spontaneous tendency to repair the cellular damage caused by carcinogens (AUBERBACH et al., 1962b).
known that the respiratory epithelium has intrinsic capacity to re-
pair preneoplastic lesions, although obviously this potential is not 
always adequate to prevent cancer if the carcinogenic insult is ex-
cessive. Unfortunately, we do not know how much damage may occur be-
fore the preneoplastic lesion becomes irreversible. However, given 
the fact that the latent period for development of invasive cancer is 
so long, there exists a unique opportunity to arrest or reverse the 
progression of malignancy if appropriate anti-carcinogenic measures 
could be applied at a suitable time during the latent period. Thus, 
any chemical that is capable of enhancing the intrinsic capacity of 
the respiratory epithelium to repair preneoplastic lesions offers a 
potential means to prevent the development of lung cancer.

In our efforts at anti-carcinogenesis, we are thus searching for a 
means to enhance a natural, physiological process rather than to in-
volve a new biological mechanism. We are seeking a means to heal pre-
neoplastic respiratory epithelium, either to arrest the progression 
of metaplastic cells or to replace these metaplastic cells with the 
ciliated and mucus cells which normally line the epithelium. While 
searching for agents that are capable of modifying the cellular state 
of tracheobronchial epithelium, one naturally turns to vitamin A, 
which normally controls the proper physiological differentiation of 
ciliated and mucus-producing cells in this epithelium (WOLBACH and 
HOWE, 1925; WONG and BUCK, 1971; HARRIS et al., 1972). It is just 
these ciliated and mucus cells that disappear from the epithelium 
during the development of squamous metaplasia induced by carcinogens 
(AUERBACH et al., 1961; HARRIS et al., 1972). Our efforts in anti-
carcinogenesis have been encouraged by the finding, in a number of 
long-term experimental studies with intact animals, that vitamin A 
or its analogs has a definite ability to prevent the development of 
SAFFIOTTI et al., 1967). The mechanisms whereby vitamin A exerts this 
protective effect in the intact animal have not yet been fully clari-
fied. Because of the complexity of studying this problem in the intact 
animal, we have chosen to study it in an organ culture system, as well 
as continuing with studies on animals.

Although our ultimate goal is the repair of metaplasia induced by 
carcinogens, to begin with we chose to study the repair of a simpler 
epithelial lesion, namely keratinized squamous metaplasia induced by 
vitamin A deficiency. If we could demonstrate healing of this simpler 
lesion in organ culture, it would increase our confidence in attempt-
ing to study healing of metaplasias induced by carcinogens with the 
same in vitro methods. Although our studies on this topic are just be-
ginning, we can report with confidence that in organ culture, vitamin 
A and its analogs can effectively and rapidly reverse keratinized 
squamous metaplasia caused by vitamin A deficiency. We have developed 
an assay that is quick, reproducible, and can be used to measure the 
biological activity of new synthetic analogs of vitamin A. Biological 
activity of retinoic acid can be measured at concentrations as low as 
10^{-9} molar, which offers a convenient standard for evaluation of any 
analogs made by the organic chemist.

Materials and Methods

The methods that have been used have been described in detail (CLAMON 
et al., 1974a; SPORN et al., 1974). In brief, we have allowed a tra-
cheal squamous metaplastic lesion to develop in organ culture before