Effect of He-Ne Laser Radiation on Adhesive Properties of the Cell Membrane

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At the present time, many experimental and clinical studies have been carried out to examine the interaction of low-power visible (especially red) laser light with tissues and cells [3,7], and their results have laid the foundation for laser therapy. However, the molecular mechanism of this interaction remains to be elucidated. At the cellular level, a mechanism of photosignal transmission and amplification has been proposed that relates light absorption by primary photoacceptors and respiratory chain components to the final macroeffect of the radiation, namely accelerated cell proliferation [6]. The proposed scheme [6] includes various responses of the cell membrane, for example those altering the ionic permeability and electric potential of the cell membrane and the activities of several enzymes.

The present study was undertaken in an attempt to develop that scheme further by examining variations with time in the adhesive properties of membranes in HeLa cells following their exposure to radiation from a He-Ne laser. Previously, this model of cultured HeLa cells was used to explore how monochromatic visible light affects the proliferation of cell populations depending on its wavelength, intensity, and dosage [2,9].

MATERIAL AND METHODS

HeLa cells were cultured in scintillation vials in medium 199 supplemented with 10% bovine serum and with antibiotics (100 units/ml each of penicillin and streptomycin). The cell monolayer was irradiated, 72 h after seeding, with a defocused He-Ne laser beam (10 J/m^2, 10 sec, 10 W/m^2) in the dark, as previously described [2,9]. The criterion for judging whether the adhesive properties of the cell membranes had been altered was the strength of cell-cell contacts, which was assessed by noting the number of solitary cells and of cell complexes in suspension using a standard procedure for dispersing the cell monolayer.

The standard dispersing procedure included the following steps: washing the monolayer once with a warm (37°C) Versen's solution, thermostating it in 1 ml of this solution at 37°C for 5 min, and then pipetting it 30 times, always with a pipet having the same diameter of the opening. This method of preparing the cell suspension with minimal damage to the cells prevented their spontaneous aggregation. The resultant suspension was placed in a Goryaev chamber and the solitary cells as well as those that had aggregated to form two- or three-cell complexes were counted. For each data point, 3 to 5 vials were used in which a total of 500 to 1000 cells were counted. Three replicate tests were run. In addition, the numbers of live and dead cells were estimated using intravital staining with trypan blue.

RESULTS

The dose of 100 J/m^2 was selected because, as found in our earlier studies, this dose of He-Ne laser radiation is optimal for stimulating the proliferation of HeLa cells [2, 9]. In the control

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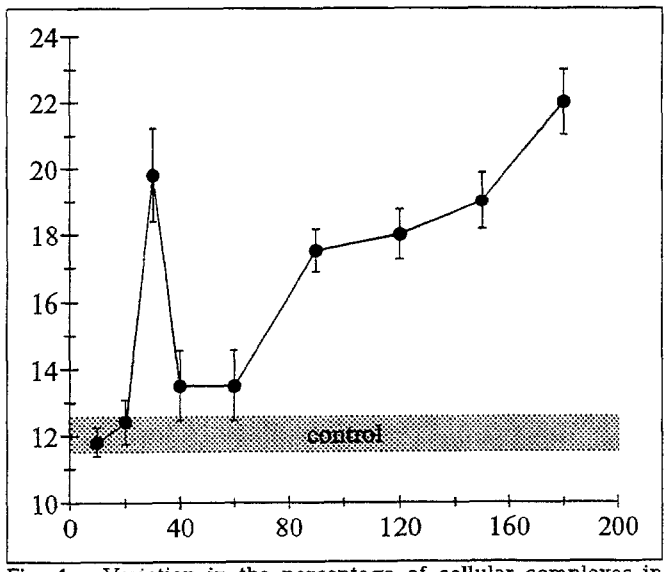

Fig. 1. Variation in the percentage of cellular complexes in suspension with the time elapsing after exposure of a cell monolayer to He–Ne laser radiation.

(nonirradiated samples of He-La cells) where the standard conditions of culturing were also used and the cell suspensions were prepared as outlined above, the proportions of solitary cells, two-cell complexes (doublets), and three-cell complexes (triplets) virtually did not vary with time, constituting, respectively, 89.6±0.9%, 7.6±0.5%, and 4.6±0.6% of all cells in the suspension. Complexes of more than three cells were not detectable in either the control or the test suspensions.

In contrast, the irradiated samples exhibited well-defined variations in the proportion of cell complexes depending on the time elapsing after the irradiation (Fig. 1). As seen in this figure, the proportion of doublets and triplets rose initially to reach its maximum at minute 30 postirradiation (19.6±1.3%), after which it fell to the control level (12.2±0.5%) before starting to rise again.

Consideration of variations in the proportion of solitary cells and of doublets and triplets (Fig. 2) showed that the proportion of doublets changed very similarly to that of triplets during the first 60 min postirradiation. Later on, however, between minutes 60 and 150, the proportion of triplets steadily rose (Fig. 2, *c*) whereas that of doublets remained virtually at the control level (Fig. 2, *b*).

These findings indicate that the short-term (10 sec) irradiation caused considerable changes in the strength of cell-cell contacts junctions over 3 h after the irradiation. The time course of alterations in the strength of these contacts was fairly complex, as is evident from Figs. 1 and 2. These alterations were not associated with overt damage to the cell membrane, as was attested by the results of intravital staining with trypan blue: the proportion of viable cells in the test and control samples was the same (91±6%).

The physicochemical and molecular bases of adhesive interactions among cells have not been fully clarified, but it is generally believed that the adhesive properties of cells depend both on the chemical composition of their surfaces and on the physical condition of the deep layers of their membranes [1,4,5,11].

Although the results of this study do not permit any conclusions to be drawn regarding the causes of alterations in the strength of cell-cell contacts, they do lead to the conclusion that the cell membrane participates in the complex chain of events involving the transmission and amplification of the photosignal in the cell after its irradiation [6]. That the cell membrane is implicated in radiation-dependent changes in cell metabolism is also supported by altered ATP [10] and cAMP [9] concentrations in cells exposed to He-Ne laser radiation. Adhesive properties of the cell membrane have been shown to depend on both the ATP [11] and cAMP [12] levels in the cell. It is also appropriate to mention here that prior laser irradiation of a HeLa cell monolayer increased the seeding efficiency if the irradiated cells

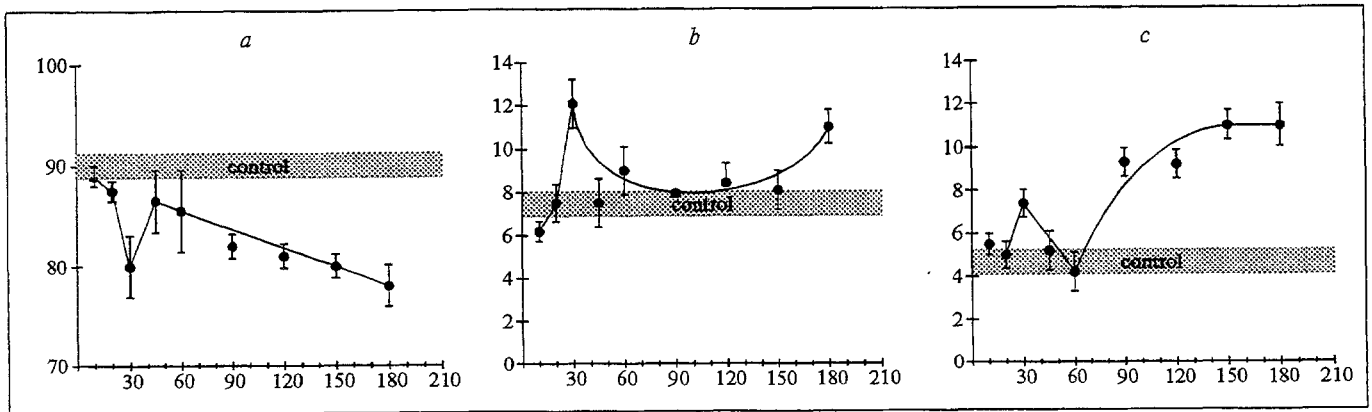

Fig. 2. Variations in the percentage of single cells (*a*) and of two–cell (*b*) and three–cell (*c*) complexes in suspensions prepared at various times after exposing a cell monolayer to He–Ne laser radiation.

were then seeded into a fresh nutrient medium [9] - a finding that is another good indication that adhesive properties are altered in laser-irradiated cells.

In summary, the present results show that a brief (10 sec) exposure of He-Ne laser radiation entails complex and protracted temporal (over 3 h) variations in the strength of intercellular adhesion.

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Primary Interaction of "Latent High-Avidity Antibodies" from Preparations of Gamma-Globulin is Probably Determined by the Presence of a Cofactor

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The presence of high-avidity autoantibodies against native DNA (nDNA), which can be revealed by the Farr test (radioimmunoassay with precipitation by 50% ammonium sulfate in the presence of a coprecipitant), is an important criterion of systemic lupus erythematosis [3, 7].

Previously we isolated a fraction of high-avidity proteins from commercial preparations of human γ-globulin, whose interaction with nDNA may be revealed with the Farr test. This property was explained by the presence of latent natural DNA-reactive antibodies in the total pool of γ-globulins which are released from the complex during ion-exchange chromatography [6].

In the present study the role of different isotypes of immunoglobulins in newly detectable nDNA-binding activity was investigated.

MATERIALS AND METHODS

Commercial preparations of human γ-globulin (or immunoglobulin) were used in the experiments. The methods for isolation of the nDNA-binding fraction and determination of nDNA-protein inter-

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