Changes in Surface Morphology of Rat Thymocytes
Accompanying Interphase Death

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Radiation-induced changes in the surface morphology of rat thymocytes in suspension were
evaluated by scanning electron microscopy. Viable (erythrosin B unstainable) cells were gene-
 rally rounded with their surface exhibiting undulations of short microvilli. Exposing the cell
suspension to 1 kR X-rays and 4 hour incubation at 37°C caused disappearance of microvilli.
Association of dead (erythrosin B stainable) thymocytes with smooth, occasionally porous, surface
without any digitations was confirmed by the examination of enriched dead cell populations.

INTRODUCTION

Mammalian thymic lymphocytes show a typical interphase death after a low dose
X-irradiation. In the preceding investigations we noticed that dead rat thymocytes,
judged by erythrosin B uptake, were dense in their buoyant densities, and as a result,
could be collected in the most dense fraction, distinctly separated from viable (non-
stained) cells, on silica sol (Percoll, Pharmacia) density gradient centrifugation1). Cell
size distribution curves demonstrated further that dead cells were smaller in cell
volume as compared with the normal viable cells2).

On the other hand, morphological studies involving light and electron microscopy
suggest that lymphocyte surface morphology is dynamic and exquisitely susceptible to
changes in cellular conditions3,4). The present investigation was undertaken to charac-
terize by scanning electron microscopy (SEM) the shape of the thymocytes irradiated
in vitro and to determine the cell surface changes that accompany the development
of the radiation-induced phenomena mentioned above.

MATERIALS AND METHODS

Detailed procedures for rat thymocyte preparations, X-irradiation, incubation and
dead cell separation by Percoll density gradient centrifugation were described previously1,2).
Briefly, thymocyte suspensions in Krebs-Ringer Phosphate (KRP) solution (pH 7.4), prepared from Wistar strain rats, were incubated at 37°C for 4 hours after 1 kR X-irradiation. The incubation time of 4 hours was chosen for the examination of the cells by SEM, because, at this time, about half of the irradiated cells became erythrosin B-stainable, which formed a distinct sub-population differing from the viable cells by smaller cell size and higher buoyant density. Enrichment of dead (erythrosin B-stainable) cell population was performed by centrifuging the cells in Percoll (Pharmacia, Uppsala, Sweden) KRP gradient medium at 11,000 g for 30 min. Dead cells were collected in the most dense fraction of the gradient. Cell viability was determined by erythrosin B exclusion test.

Incubated or separated cells were attached to poly-L-lysine coated glass coverslips, and fixed with 1.0% glutaraldehyde in KRP, washed, and then post-fixed in 1.0% aqueous osmium tetroxide, washed and dehydrated through a graded series of alcohol followed by isoamyl acetate five minutes each and dried at the critical point of CO₂ in a JOEL JCPD-3 critical point drying apparatus. The coverslips were mounted on stubs and coated with ca. 200 Å of gold with a JFC-1100 ion sputtering apparatus. Samples were viewed with a JEOL LSM-F7, field emission type scanning electron microscope operated at 7 kV.

RESULTS

After examination of more than several hundreds cells, it appeared that the majority of the unirradiated thymocytes (control) exhibited moderately undulating surface with some degree of irregularity showing digitations (Fig. 1). These digitations were quite similar to those described for T lymphocytes and were either stub-like or short microvilli.

Fig. 2 illustrates surface features of the cells incubated for 4 hours after irradiation. Appearance of the cells possessing relatively smooth surface without digitations and a clear decrease in number of cells having microvilli are obvious, although a spectrum of surface architecture was seen. The occurrence of a high proportion of the cells devoid of digitation in the irradiated sample suggests that radiation-damaged cells were those with microvillus-deleted surface architecture while surviving cells corresponded to those with more undulating surface with short microvilli.

This was also evident from experiment where the dead (erythrosin B stainable) cells were obtained in a concentrated form after separation from viable cells using Percoll density gradient; the cells without digitation were also concentrated (Fig. 3). The vast majority of these cells, collected from the most dense fraction of Percoll density gradient, had smooth, slightly irregular surface without digitation. Some cells had a moderate number of surface fold, openings or holes of various size. Erythrosin B uptake test revealed that more than 90% of these cells were "dead". It is likely from these results that erythrosin B stainable cells, which had a higher buoyant density, corresponded to the cells without surface digitation.
Fig. 1. A low power micrograph revealing the general architecture and distribution of normal unirradiated thymocytes in suspension. Most of the cells are rounded and reveal microvilli evenly distributed on their surface. Marker equals 10 μm (x3000).

Fig. 2. Cell suspension incubated at 37°C for 4 hours after 1 kR X-irradiation. Approximately half of the cells have smooth, but slightly irregular surface devoid of microvilli. Intact cells are also seen. Marker equals 10 μm (x3000)
Fig. 3. "Dead" cell enriched population (collected from the most dense fraction of Percoll density gradient). Most of the cells are spherical with irregular surface without digitations. Intact cells are only occasionally seen. Marker equals 5 μm (×5000).

Fig. 4. "Viable" cell enriched population (collected from more buoyant fraction of Percoll density gradient). The majority of the cells have short microvilli even after 4 hour incubation following 1 kR irradiation, same appearance encountered in non-irradiated viable cell suspension. Marker equals 5 μm (×5000).
Making striking contrast to these cells, thymocytes shown in Fig. 4, which were collected from more buoyant fraction of Percoll density gradient and proved viable (non-stained) even after irradiation, exhibited the stub-like microvilli projecting from their surface, characteristic surface feature of viable non-irradiated cells.
It is reasonable to conclude from these findings that the cells having no digitations or microvilli, may represent "dead", i.e. erythrosin B stainable cells, while "viable" (non-stainable) cells were characterized by villous surface architecture showing stub-like digitations or short microvilli.

High magnification of "dead" cell surface morphology are shown in Figs. 5 and 6. Presence of generally smooth surface without digitations are clearly visible. Openings or holes in the membranes are occasionally observed. Size and number of these holes varied from cell to cell.

DISCUSSION

A large accumulation of information is available concerning cytological and biochemical changes accompanying interphase death\(^7\), however, there has been little detailed investigation of the associated surface morphology. Evidence for the association of disappearance of microvilli with dead rat thymocytes was provided in the present study by the examination of enriched dead cell population. Loss of microvilli appears to be one of the first responses of the cells to an unfavourable environment, perhaps to reduce the exposed cell surface\(^7\). The characteristic surface of dead or dying cells was also obtained when the lymphocytes were incubated with cytotoxic agents\(^7\). Our present findings on rat thymocytes damaged by radiation are consistent with the above results. Mechanisms for the radiation-induced changes in surface morphology remain to be investigated, although damages to cytoskeleton systems are probably involved in the mechanisms underlying loss of microvilli.

The term "dead" or "viable" is, however, in this paper used merely as a convenience in nomenclature, since cell death in interphase is difficult to define experimentally, because it is a progressive phenomenon and a history of the intermediary phase between perfect life and unquestioned death. Previous studies in our laboratory have shown that erythrosin B uptake might be a reflection of a definite intermediary phase within the progressive death process, and the cells which is stainable with the dye, have a higher buoyant density and a discrete cell size smaller than the normal viable cells\(^1\). The present SEM study demonstrated further that erythrosin B stainable cells are distinctly different in surface morphology from the normal viable cells; loss of microvilli occurs concomitantly with changes in membrane permeability for dye, and in buoyant density as well as cell size.

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