

INTERNUCLEAR INTERACTION BETWEEN EFFECTOR AND TARGET CELLS IN CELL-MEDIATED IMMUNE REACTIONS

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The mechanism of cytotoxic cell-mediated lesion of target cells (TC) is not yet definitely ascertained. It mainly concerns cytotoxic T-lymphocytes (CTL) and natural killers (NK), which are able to kill spontaneously tumoral and infected cells [1, 2, 3]. The effect of the cells killers is regarded as one of the mechanisms of recovery at tumors and many viral infections, due to clearing of the organism from pathologic cells by their destruction [4].

It is known that for the injury of TC a cellural contact is necessary for starting of the lytic mechanism of effector cells (EC). There exist different hypotheses and facts explaining some aspects of this process [5]. A significant attention is paid to the secretory function of EC [6], cytolytic components of CTL granules [7], migrating in the direction of the target. Some importance is given to the cytotoxic factor of NK cells (NKCF) [8, 9], mobilization of CTL calcium, to the rearrangement of the cytoskeleton-associated Golji apparatus, discharge of soluble lymphokins CDH+ by the cells [14] or destabilization of lysosomes. The morphologic investigations carried out by Malorni et al. (1989) made him to suggest a hypothesis of the "suicidal behavior" of the target cells in NK of cell-mediated lysis.

There are found pre-forming molecules of perforin, discharged by EC, able to damage directly the TC membranes [6].

Young & Cohn (1987) though attach some importance to the role of perforin in the mechanism of the cell-lysis, do not exclude the possibility of existence of other ways of target killing.

For destruction of the cell the main index is a nuclear lesion. By Russel et al. [10] the change in TC was observed at the early stages as a destruction of DNA into small fragments due to the rupture of the nuclear membrane. After the "lethal hit" inflicted by CTL in TC an induced nuclear lesion is observed with following "internal disintegration" of the nucleus and its further destruction into small fragments.

The existing hypotheses and facts are based mainly on the data obtained *in vitro*. Parallel investigations of the cellular kinetics *in vivo* in conditions of a whole organism as well as in patients will allow to solve this problem.

Thus, in the given work I try to present all those peculiar changes, which are observed in EC in interaction with TC at some pathologic processes in human-beings, using the surgical and biopsy material. The data were collated with the results of the experimental investigation of rats after intraperitoneal administration of complete Freund's adjuvant and B group streptococcal culture.

Material and methods

42 ordinary male albino rats with weight 200-220 g were injected intraperitoneally 0,1 ml of Freund's adjuvant (Difco, Lab. Detroit, Michigan, USA). The control group consisted of 6 animals. The rats were decapitated after 1, 2, 3, 4, 6, 12, 24 hours and 3 and 7 days. Membranous preparations from the mesentery on the slides and imprints from the surface of the spleen cut, which were fixed in acetone at 4°C during 15 min were prepared. The slices of the spleen were fixed in acetone during 1 hour. The paraffin sections, imprints and membranous preparations were stained by ordinary histologic methods, Feulgen's reaction on DNA was put.

The method of investigation of the membranous preparations of the rat mesentery cells enabled to study the tissues layer by layer on a large area, where the cellular and tissue elements were observed in plane arrangement without disturbing their form and interaction. The method allows to obtain a whole notion of the cells and their processes, which are in the tissue on different levels. Frequently met fibrous structures, ensuring the contacts between cells on a distance, have usually a form of short stretches on sections. It is practically impossible to reconstruct the three-dimensional picture of the cells' interaction by thin sections. The membranous preparations in light microscopy allow to obtain information about whole cells and their processes in the three-dimensional picture, in natural cellular co-operation, in conjugates in interconnection with the microsurrounding.

Parallely, 20 male albino rats were injected intraperitoneally with B group streptococcal culture (090 R strain) in the dose 6×10^8 microbial bodies in 1 ml of sterile physiologic solution. The animals were killed 1 and 3 months after the infection. The knee joints were studied with a scanning electron microscope (SEM). After removing the skin and subcutaneous fat the cavity of the knee joint was opened. The object was fixed in 2.5% solution of glutaraldehyde on 0.1 M phosphate buffer, pH7, at 4°C during 1-2 hours, then for three times was washed in phosphate buffer during 15 min and after it lyophilized. The surfaces of synovial membranes and from the suprapatellar pocket, medial and lateral walls of the joint capsule were studied with SEM "Jeol JSM-35C" after spraying in "Jeol Finecoa JEM-1100" sprayer.

For transmission electron microscopy (TEM) small bits of the spleen were fixed in 2.5% solution of quadric oxide of osmium. After dehydration in spirits of increasing concentration the bits were put in araldid. From each block there were prepared semithin sections with 1 μm thickness, going along the whole area of the bit. On the basis of those sections ultrathin sections were prepared, contrasted by uranyl acetate and lead citrate and were studied under the microscope. Semithin sections were stained with Methylene Blue.

MESENTERY. In membranous preparations 1 h after intraperitoneal administration of Freund's adjuvant it is observed a moderate inflammatory reaction with the presence of plural neutrophilic leukocytes, macrophages and lymphocytes. After 4 hours in the exudate parallel with leukocytes there are found out groups of cells with spherical and elongated nuclei. It is peculiar the appearance of cells with comma-shaped nuclei during 1-2 h, which during next days are revealed in form of focal and diffuse accumulations.

There are met effector: target (E:T) conjugates with lysis of TC nuclei. As EC the lymphocytes with spherical and elongated nuclei react in a close contact with the nucleus of TC.

1 h after the administration of Freund's adjuvant in the connective tissue there appear long, up to 500 μm , basophilic fibers, coming out of EC nuclei.

Most of them have contact with TC nuclei by their terminal end. The fibers are not stained on fibrin by Weigert and show weak PAS-positive reaction. The initial part of the fiber has a narrow layer of cytoplasm, which disappears at the terminal part. Within the structure there are no organellas, but in case of both electron and light microscopy most of the fibers are not homogeneous. In some of them the axal Feulgen-positive part is electron-dense, in others it consists of thin parallel fibers of chromatin. The connection with EC nucleus, basophilia and positive reaction on DNA allow to consider them as chromatin fibers (CF).

The nuclei, giving off CF, have different shapes. In one case they are bean-shaped or spherical, give off usually two CF (fig. 2), in other case they are spindle-shaped, one end gradually lengthening and becoming a thin and long CF. Some have nuclei with a shape of a canopy and thin fibers of chromatin stretching and gathering into one long CF.

Light microscopy enables to reveal two morphologic variants of CF:

- 1) fibers with the length up to 100 μm with wide and narrow areas along their length. The fibers, consisting of gradually connected 2-3 blocks of chromatin, the medium parts of which are significantly thin, are very interesting. In the area of the connection of blocks the chromatin is mainly decondensated and shows a weak Feulgen-positive reaction.

The terminal from EC block is the most thin, the end is sharp, acerous. Sometimes its end has a granule from compact chromatin;

- 2) fibers which are interrupted, like a thin homogeneous thread from chromatin with a length up to 800 μm . Gradually becoming thinner, the CF end becomes sharp or has a granule of chromatin.

Some of them are in contact with the TC by their terminal parts and resemble a taut string between the nuclei of two cells.

The CF, coming out of the nucleus in 2 or more are evenly thin and don't have a block construction.

Dependent on the interaction with the surrounding cells in the connective tissue, there are revealed two structural-functional varieties of ECF: fibers in contact with the nuclear membrane of TC and fibers with free ends. More often the CF begins from the peak of the cone-shaped nucleus of EC and its terminal part ends on the nuclear membrane of TC. In absence of contact with TC nucleus the CF is fibrous, has curves, passing at the neighbouring cells, and usually does not have a granule of chromatin on its end.

Judging by the morphologic signs one may say that the CF are the forms, that come out of TC after the "lethal hit" and their fate in future is unknown. It is necessary to carry out auxiliary investigations in order to find out the answer. Nuclei, which give off 2 or more CF are not often met. They appear 6 hours after the beginning of the experiment and are observed during 6 days. Their chromatin is homogeneous, compact, shows Feulgen positive reaction and is stained intensively by hematoxylin.

There are observed as well cells with three or four long, up to $150\ \mu\text{m}$ ECF, having a shape of processal cells. The nucleus giving off three CF is triangular, has a shape of neuron with long processes, consisting of nuclear substance. In case of presence of four or more CF in the place of the nucleus it is revealed an intensively stained granule from compact chromatin.

SPLEEN. In the prints, taken from the surface of the spleen sections after a single administration of the adjuvant in all rats after 3 hours up to the third day there are determined E:T conjugates with intercytoplasmatic and internuclear ponticuli. In the composition of the conjugates there are met three morphologic varieties of killers:

- 1) lymphocytes with spherical nuclei;
- 2) cells with elongated or pear-shaped nuclei, which in the cellular conjugate have contact with the nuclei of one or two targets;
- 3) elongated cells with comma-shaped nuclei with one sharp aceros end.

In semi-thin sections there are well observed cells, which have contact with simultaneously 2-3 lymphocytes. In these E:T conjugates the nuclei of TC are intensively stained, chromatin is compact and closely adjoins cytoplasm of the TC nucleus. Such TC are unchanged in condition of karyolysis or sometimes, karyorrhexis.

By their morphologic signs during two hours EC killers are lymphocytes and cells with elongated nuclei.

Subsequently, there appear plural cells with comma - shaped nuclei with sharp ends. In a role of the target act mainly the lymphocytes with spherical nuclei.

In all terms of the experiment beginning from the first hour and during 7 days the contact of such EC with the target lymphocyte ends in cell-mediated lympholysis.

The mechanism of the damaging effect of a cell with a comma-shaped nucleus of the lymphocyte consists of a few stages. The cellular interaction begins with adhesion of the killer with the lymphocyte.

Gradually, EC envelops TC in a form of a half-moon. When the ends of the comma-shaped nucleus meet and its both poles merge around the lymphocyte a "nuclear ring" is formed. The narrowing of the ring of EC, lysis of the cytoplasmic membranes in the area of the contact is accompanied by merging of the nuclei of both cells. The process ends in lysis of the nucleus of the lymphocyte in "embrace" of the EC nucleus. A similar picture is frequently observed with neutrophilic leukocytes and macrophages. The cells with comma-shaped nuclei co-operate with the TC nucleus having a sharp end as well.

The activation of the cells with comma-shaped nuclei under the influence of the adjuvant is accompanied by the appearance of extremely long elements forming CF.

Such elements co-operate with TC nucleus not only by a ring-shaped embrace with a long CF, but by the terminal end too. The contact takes place on the level of the nuclear membrane of the TC, into which the end of the CF penetrates through the cytoplasm of the cell.

Nucleus to nucleus interaction in the E:T conjugates of the spleen is found also in the contact of the cells on the level of the cytoplasmic membranes. On semithin sections between the nuclei of the interacting cells it is revealed a chain of chromatin granules, connecting the nuclear membranes.

EXPERIMENTAL STREPTOCOCCAL ARTHRITIS. In the knee joints of the rats 15 days after a single intraperitoneal administration of E group streptococcal culture there are revealed destructive changes of the articular cartilage and moderate inflammatory reaction of synovial membranes.

There are observed signs of proliferation of synoviocytes. By SEM between the fibers there are revealed plural long intercellular ponticuli. Morphologically they are identical to filopodia of EC in contact by their terminal end with synoviocytes of the target. On the third month synovial membranes are acutely stripped, sclerosed, synoviocytes are scarcely observed and, there are observed intercellular ponticuli with destructive changes of synoviocytes. At the same time in the freeze-etch images of the joint capsule there are observed plural large cells in contact with lymphocytes and extracellularly persisting streptococci.

For the study of the morphology of EC killers in the process of their interaction with TC and revelation of the mechanisms of the cytotoxic effect an experimental investigation was carried out.

It was established that in the connective tissue there are revealed interacting cells, forming E:T conjugates. The main morphologic sign of the cellular reaction is a close contact of the EC nucleus with the TC nucleus. During the contact the nucleus of the killer cell gives a "lethal hit" to the TC nucleus. These data are new and have never been described before by other authors. Only J. Russel et al. [10] expressed the

idea that an agent, causing a nuclear lesion of TC is initiated through CTL. The character of this agent is not known yet.

There are observed as well long fibers of chromatin, stretched out from the nuclei of separate EC, contacting by their distal ends with the TC nucleus on a distance.

The adhesion with TC, its embrace in a form of a half-moon, formation of lysating "nuclear ring" of EC around the lymphocyte and merging of nuclei after the lysis of cytoplasmatic membranes end with karyolysis of the TC nucleus. This process in its dynamics is described for the first time, and it is a morphologic documentation of cell-mediated immune lympholysis.

In this case the EC nucleus acquires a form of a stretched spindle with 50-60 μm length with some thickening in the middle. These cells morphologically are like elements described by G.Grossi et al. [11], Malorni et al. [12] in cell culture at investigation by SEM. Human T lymphocytes bearing CD3-associated T-cell receptors form a distinct uropod, and in the final phase of adherence, omit long filopodia ending with adhesion plaques. According to my data, the basis of filopodium is a nucleus, which stretching in a form of CF ends with a granule of chromatin or button-like formation of Feulgen- positive substance on the terminal end.

The appearance of the cells with comma-shaped nuclei under the influence of the adjuvant in the experiment is the result of cytodifferentiation, directed towards insuring of the realization of the specialized cellular function. The cells with comma-shaped nuclei realize a "lethal hit" to the nuclei of the TC with their stretched ends or with CF. The stretched end in a shape of a short CF often completely wraps macrophages and neutrophilic leukocytes. Frequently there are observed two CF from both poles of the nucleus, where the cell interacts simultaneously with two targets.

The destruction of TC takes place not only in result of the nucleus lysis, but because of karyopyknosis, karyorrhexis and apoptosis. Disintegration of a nucleus with DNA fragmentation develops, which according to A.Wyllie [7] is connected with activation of the nuclear endonuclease.

The structural analysis of cellular interactions in the spleen and connective tissue of the rat after administration of Freund's adjuvant in streptococcal arthritis show that CMIR is accompanied by stimulation of two populations of EC. One population shows the quality of the killer, realizing the cytotoxic effect on TC by contact to the nuclei. The second population has a double function on the base of the informative and destructive cellular reactions. Affecting directly the nucleus of EC as a killer, simultaneously it gives information to the nucleus of another killer through CF. These contacts in the spleen tissue take place by transcytoplasmatic transmission of chromatin granulations in a form of a chain from the nucleus of one cell to another in their contact on the level of cytoplasmatic membranes.

The structural information of cells, as a manifestation of cellular reactions, takes place in limits of the connective tissue, which is a self-regulating system functioning in norm and pathology on the base of the inverse connection [13]. In other words it is a matter of immunologic control in the focus of inflammation.

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ԷՖԵԿՏՈՐ ԵՎ ԹԻՐԱԽ ԲԶԻԶՆԵՐԻ ՄԻՋԵՎ ՄԻԶՎՈՐԻԶԱՅԻՆ ՓՈԽԱԶԴԵՑՈՒԹՅՈՒՆԸ ԲԶԻԶՆԵՐՈՎ ՄԻՋՆՈՐԴԱՎՈՐՎԱԾ ԻՄՈՒՆԱԲԱՆԱԿԱՆ ՌԵԱԿՑԻԱՆԵՐԻ ԺԱՄԱՆԱԿ

Ն. Դ. Վարդազարյան

Փորձի պայմաններում առնետների մոտ ուսումնասիրվել է էֆեկտոր եւ քիրախ բջիջների փոխհարաբերության ձեւաբանությունը, բջիջներով միջնորդավորված իմունաբանական ռեակցիաների (ԲՄԻՌ) ժամանակ:

Ֆրեյնդի ադյուվանտի ներդրվայնային ներարկումից հետո 24 ժամվա ընթացքում ուսումնասիրվել են կենդանիների փայծաղի եւ միջնդերքի թաղանթների պատրաստուկները: Էլեկտրոնային մանրադիտակով հետազոտվել են Բ խմբի ստրեպտոկոկով վարակված կենդանիների ծնկան հողի սինովիալ թաղանթները:

Հայտնաբերվել է բջիջների միջեւ միջկորիզային փոխազդեցության հետաքրքիր փաստ, որն ընկած է ԲՄԻՌ- հիմքում: Էֆեկտոր բջիջը իրականացնում է իր «մահացու հարվածը» քիրախ բջջին իր կորիզի միջոցով: Մի դեպքում այն կատարվում է երկու բջիջների կորիզների անմիջական հպման միջոցով, մյուս դեպքում՝ քրոմատինային թելերի օգնությամբ, որոնք արձակվում են էֆեկտոր բջիջների կորիզների կողմից տարածության վրա:

Կախված էֆեկտոր բջջի կորիզի ֆունկցիոնալ ակտիվությունից, նա կարող է միաժամանակ փոխազդեցության մեջ մտնել մեկից մինչեւ յոթ քիրախ բջջի հետ:

Այս մեխանիզմը ունիվերսալ է, գործում է շարակցական հյուսվածքի եւ իմուն համակարգի բջիջների միջեւ տեղեկատվության հաղորդման համար:

МЕЖЪЯДЕРНОЕ ВЗАИМОДЕЙСТВИЕ МЕЖДУ ЭФФЕКТОРНЫМИ КЛЕТКАМИ И МИШЕНЯМИ ПРИ КЛЕТОЧНО-ОПОСРЕДОВАННЫХ ИММУННЫХ РЕАКЦИЯХ

Н.Д. Вардазарян

В эксперименте на крысах изучалась морфология эффекторных клеток в условиях взаимодействия с клетками-мишенями при клеточно-опосредованных иммунных реакциях (КОИР).

После однократного внутрибрюшинного введения адьюванта Фрейнда изучались препараты из селезенки и пленки из брыжейки. Сканирующим электронным микроскопом исследовали синовиаль-

ные оболочки коленного сустава крыс, инфицированных В-стрептококком.

Выявлен интересный факт межъядерного взаимодействия между эффекторными клетками и клетками-мишенями, лежащий в основе КОИР.

Эффекторная клетка осуществляет "летальный удар" посредством своего ядра. В одном случае это осуществляется путем непосредственного контакта обоих ядер, в другом — посредством хроматинового волокна, которое испускается из ядра эффекторной клетки на расстоянии. В зависимости от функциональной активности эффекторной клетки ее ядро одновременно может взаимодействовать с ядрами до 7 клеток-мишеней.

Данный механизм универсальный, действует также для передачи информации между клетками соединительной ткани и иммунной системой.

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