REVIEW

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BIOCHEMICAL AND CELLULAR MECHANISMS OF IMMUNOGENIC CELL DEATH

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Immunogenic cell death (ICD) is a mode of programmed cell death that leads to the activation of anticancer immune response and determines the long-term success of anticancer therapies. Here, we provide a review of the known molecular and cellular mechanisms of ICD. Usually, solid tumor experimental models have been used in ICD studies. However, ascites tumor models may possess some advantages over them. The results of our investigation on the approbation of murine Nemeth-Kellner lymphoma as an experimental ascites tumor model for ICD studies are presented.

K e y w o r d s: immunogenic cell death, biochemical mechanisms, ascites tumor model, murine Nemeth-Kellner lymphoma, doxorubicin, oxaliplatin.

In contrast to apoptosis, the most studied type of regulated cell death, which has long been regarded as non-immunogenic and tolerogenic [1], ICD is accompanied by a release of damage-associated molecular patterns (DAMPs). These molecules promote antigen presentation and phagocytosis, eventually activating innate and cognate immune responses. This response is targeted against dying cells and their viable counterparts from the same population with the same antigenic profile.

Immunogenicity is also a key feature of necrosis and necroptosis. However, in contrast to necrosis, ICD is a regulated process. Besides this, some steps of DAMPs release in ICD are caspase-dependent, relying in particular on caspase-8 (CASP-8), an initiator caspase of an extrinsic apoptosis pathway [2]. Considering this and other factors, ICD has sometimes been defined as an immunogenic apoptosis [3, 4]. However, this definition is not entirely accurate. Some other modes of regulated cell death, such as necroptosis, pyrrhoptosis, and ferroptosis, have demonstrated the patterns of DAMPs release that are characteristic of ICD [5]. It has been shown that some agents that usually induce apoptosis in tumor cells can induce necroptosis or pyrrhoptosis in other types of cancer cells. The relationship between ICD and apoptosis has yet to be defined completely. Identifying more links between these processes would help repurpose apoptosis agents for the ICD induction.

Various factors, both chemical and physical, can induce ICD. Among physical ICD-inducing factors are heat shock, high hydrostatic pressure and ionizing radiation. ICD induction is also responsible for the anticancer effects of hypericin-based and redaporfin-based photodynamic therapy [6-9]. In the latter cases, ICD is induced by a combination of physical and chemical factors that cause ROS production and ER stress in cancer cells. Many chemical factors that induce ICD have been used in cancer chemotherapy long before their ICD-inducing properties have been found. The common examples are doxorubicin and oxaliplatin (but not cisplatin). However, more chemotherapeutics have been found to induce ICD. They include bleomycin, cyclophosphamide, cardiac glycosides (digoxin, digitoxin, ouabain and lanatoside C), shikonin, anthracyclines (apart from the aforementioned doxorubicin, idarubicin and mitoxantrone have ICD-inducing properties as well) [6].

Depending on the mechanism of their action, ICD-inducers have been divided into two types.

Type I ICD inducers act by causing collateral ER stress and inducing ICD-associated release of DAMPs indirectly, without triggering ROS production. The ER stress caused by Type I inducers is by itself non-lethal. ICD inducers of Type II induce ER-stress-dependent cell death by selectively targeting ER [10]. They cause ROS production and oxidative stress or trigger the phosphorylation of the translation factor eIF2α in a PERK-mediated manner. Type I inducers include chemotherapeutics such as mitoxantrone, doxorubicin, oxaliplatin, and cyclophosphamide. A typical example of type II ICD inducer is hypericin-based photodynamic therapy (Hyp-PDT).

Molecular markers of ICD

Immune response can be activated by either non-self antigens or by the autoantigens released from the damaged cells [11]. The second case is responsible for the immunogenic nature of ICD. When tumor cells undergo ICD, they release a repertoire of signals called damage-associated molecular patterns (DAMPs) [5]. These are intracellular molecules with conventional functions inside the cell. However, when they are released out of the cell, they gain immunogenic properties [1].

These molecules can be either exposed on the cell surface or released into tumor microenvironment (TME) and trigger an immune response against tumor cells [12]. Among the secreted and released DAMPs are extracellular ATP and HMGB1 – a non-histone chromatin-binding protein. DAMPs like calreticulin (CRT), HSP70, and HSP90, are exposed on the cell membrane [13].

Based on their expression and functions, DAMPs can be divided into constitutive (cDAMPs) and inducible (iDAMPs) [1].

DAMPs can act as modulators of antigen presentation and phagocytosis by dendritic cells and macrophages. They also contribute to a proinflammatory environment facilitating innate immune response against the tumor [14]. The mechanism of such modulation involves the interaction of DAMPs with their specific pattern recognition receptors (PRRs). These are membrane receptors located on the dendritic cells, monocytes and macrophages [1], as well as natural killer (NK) cells [15]. The interaction of PRRs with DAMPs promotes the maturation of these innate immune cells. In turn, these cells can modulate and trigger adaptive immunity [16].

Although most DAMPs stimulate an immune response, some of them, e.g. adenosine and prostaglandin E2, act as immunosuppressors and induce immune tolerance towards dead tumor cells [17, 18]. The repertoire of DAMPs elicited by different ICD inducers may differ [18], but the three DAMPs, CRT, HMGB1, and ATP, are involved in ICD pathways triggered by most or almost all studied ICD-inducing factors. The externalization of CRT, together with the release of HMGB1 and ATP, are considered the hallmarks of ICD and play an important role in the activation of innate and adaptive antitumor response (Fig. 1). The following sections describe the most studied constitutive DAMPs that have critical roles in the process of ICD.

Calreticulin and its externalization in ICD. Calreticulin (CRT) is a 46 kDa soluble Ca^{2+}-binding protein with a highly conserved sequence. Under normal conditions, it resides in the lumen of the endoplasmic reticulum, where it acts as a chaperone. CRT has other functions both inside and outside the ER, such as regulating calcium homeostasis and assisting in the assembly of MHC-I complexes [19-21].

When ICD is induced, CRT is translocated from the perinuclear ER onto the outer leaflet of the cell membrane [22]. Such externalized CRT (ecto-CRT) acts as a potent “eat-me” signal [23]. Ecto-CRT facilitates the engulfment of dying cancer cells and their fragments by dendritic cells (DCs) and macrophages, providing them with an abundant source of tumor-associated antigenic material [24]. Presentation of tumor antigens by these antigen-presenting cells eventually leads to the activation of cytotoxic CD8+ lymphocytes (CTLs) and adaptive immune response against the tumor [23].

The function of ecto-CRT as an “eat-me” signal was first demonstrated by Gardai et al. [25]. Further studies by Obeid et al. showed that CRT externalization was a key determinant of antitumor immune response associated with ICD. It was demonstrated that a knock-down of CRT or its inhibition by CRT-specific antibody treatment significantly suppressed the phagocytosis by DCs [1, 26].

The molecular mechanism of ICD-associated CRT externalization includes the following stages: activation of ER-stress kinase PERK leads to the phosphorylation of translational factor eIF2α and translation arrest. This causes partial activation of pro-apoptotic caspase 8 (CASP-8), cleavage of B-cell receptor-associated protein 31 (BCAP31) and

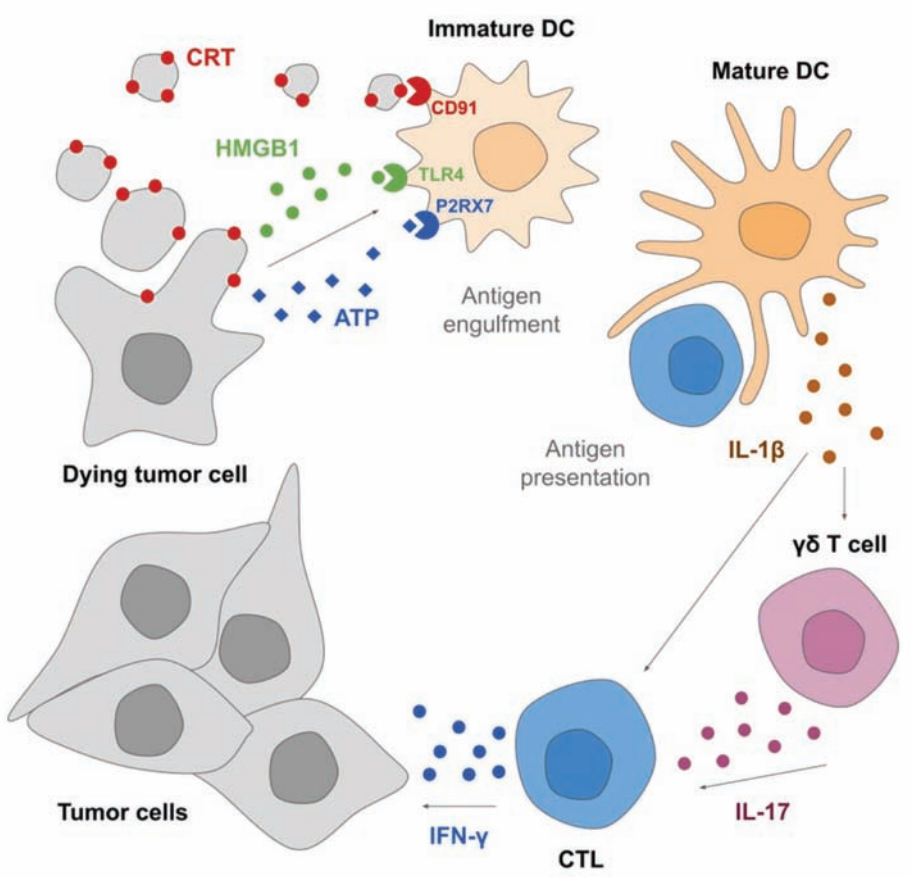

Fig. 1. The general mechanism of antitumor immune response triggered by ICD. Therapy-induced stress leads to the exposure and release of DAMPs by dying cells, including calreticulin (CRT), high mobility group box 1 (HMGB1) and adenosine triphosphate (ATP). The binding of these DAMPs to the respective pattern-recognition receptors (including but not limited to CD91, TLR4, and P2RX7) promotes the maturation of dendritic cells (DCs), enhances antigen presentation and phagocytosis by DCs and macrophages, triggers the release of cytokines and activation of cytotoxic T-lymphocytes (CTLs). Adapted from [23]

activation of BCL2-associated X protein (BAX) and BCL2-antagonist/killer 1 (BAK1). Before externalization, CRT forms a complex with another ER luminal protein, ERp57. This complex is exported from ER to the Golgi apparatus and exposed on the plasma membrane via SNARE-dependent exocytosis. Some stages of CRT externalization may vary depending on the ICD inducer [27].

Ecto-CRT is recognized by the LDL-receptor-related protein 1 (LRP1), also known as CD91. This PRR is expressed by the DCs and other antigen-presenting cells [25]. CD91-mediated signaling promotes the engulfment of cellular fragments derived from dead tumor cells. CD91 signaling pathway requires GTPase Rac family small GTPase 1 (RAC1) [25, 28].

Besides ICD-induced exposure, tumor cells exhibit a detectable basal level of ecto-CRT. A possible reason for this is that cells experience ER stress due to the oncogenic malignant transformation itself [29]. This alternative trigger of ER stress can also lead to CRT externalization and danger signaling in the absence of chemotherapy [29], thus facilitating anticancer immunosurveillance [30].

Apart from impacting CTL-mediated adaptive immunity, recent studies demonstrated that CRT externalization can also improve innate antitumor immunity by activation of natural killer (NK) cells. This activation is mediated by interleukin 15 (IL-15) trans-presentation [31].

Overall, CRT is important for the immunogenicity of ICD because its externalization promotes

phagocytosis, antigen presentation and subsequent activation of antitumor immune response.

HMGB1 release in ICD. High mobility group Box 1 (HMGB1) is a non-histone chromatin-binding nuclear protein, a member of the high mobility group (HMG) subfamily. It is expressed in all nucleated cells and is among the most abundant non-histone chromatin proteins [23]. Under normal conditions, it regulates chromatin structure and transcription. In particular, HMGB1 regulates the activity of transcriptional factors like p53, NF-κB and some others [12, 32].

During ICD, HMGB1 can be released into the intercellular medium in two ways: active secretion and passive release. It can be actively secreted via nucleo-cytoplasmic translocation and vesicular transport as the response to stress stimuli by the cells that are still viable [5, 23]. At the later stages of cell death (late apoptosis, post-apoptotic necrosis) permeabilization of nuclear lamina and plasma membrane enables passive diffusion of HMGB1 out of the dying cell [33, 34].

When HMGB1 is released passively in large quantities from the necrotic cells or cells at late stages of the ICD, it acts as a potent inflammatory mediator [35]. It promotes phagocytosis by the macrophages [36]. Similarly to CRT, HMGB1 can also stimulate antigen processing and presentation [37]. It promotes the migration of the immune cells and their infiltration into tumors [38].

Importantly, the HMGB1 release itself is not indicative of ICD only. It was documented that many drugs that are classical apoptosis inducers can trigger HMGB1 release [39]. This evidence seems to contradict other findings about proinflammatory effects of HMGB1 in ICD because apoptotic cell death is characterized as non-immunogenic. However, the immunomodulating activity of HMGB1 is largely dependent on its redox status [40]. It has been proposed that caspase- and ROS-dependent mechanisms of apoptosis are responsible for oxidative inactivation of HMGB1 [23]. This explains the apparent contradiction in the evidence above about HMGB1 in immunogenic and non-immunogenic cell death. Despite the role of HMGB1 in ICD, its release alone is not yet a reliable ICD marker [2]. However, it can be used as a marker of cell membrane permeabilization [41] HMGB1 can also be secreted in lesser quantities by viable stressed cells with relatively intact plasma membranes [5, 23]. However, most HMGB1 is accumulated in extracellular space at the late stages of cell death when the cell membrane is damaged [22, 35, 42].

HMGB1 binds to various PRRs, including TLR2, TLR4 and RAGE [43]. TLR4 is expressed on DCs and macrophages. Upon interaction with HMGB1, it triggers a signaling pathway that involves MyD88 - an innate immune signal transduction adaptor [44, 45]. TLR4-MyD88 signaling promotes antigen processing and presentation by DCs [46, 47] and is required and sufficient for immunogenicity of cell death [18]. It has been proven using pre-clinical *in vivo* models that knockout of the HMGB1 gene in cancer cells and blocking the TLR4 receptor in the host by specific antibodies reduce the immune response in animals treated with ICD inducers [46, 48]. In patients with breast cancer who received ICD-inducing anthracyclines, the loss-of-function polymorphisms in TLR4 gene were associated with unfavorable disease outcomes [46].

TLR4 signaling in macrophages can also activate the NF-κB signaling pathway that increases the expression of inflammatory cytokines and promotes their phagocytic activity [36]. HMGB1 can also activate the PI3K/AKT/mTOR signaling pathway that enhances antigen presentation in DCs and macrophages [37]. When binding to the receptors on endothelial cells, HMGB1 increases the expression of ICAM-1 and VCAM-1, leading to the enhanced adhesion, migration and infiltration of immune cells into tumors [38].

RAGE is a multiligand receptor that can interact not only with HMGB1 but also with a wide spectrum of other DAMPs, such as glycated proteins. RAGE signaling in DCs is crucial for their maturation and migration [43].

Altogether, despite HMGB1 release alone is not a determining characteristic for ICD, it plays a vital role in this process by creating a proinflammatory environment.

Extracellular ATP in ICD. Besides its normal function as an immediate energy source for almost all cellular processes, ATP can act as a danger signal when released into the extracellular medium. During ICD, extracellular ATP (eATP) acts as a chemoattractant for immune cells and has proinflammatory effects.

The mechanisms of ATP release depend on the ICD inducer. For oxaliplatin- and mitoxantrone-induced ICD, the ATP is released in an autophagy-dependent mechanism [49]. If the proteins required for autophagy, such as ATG5, ATG7 and BCN1,

are depleted, ATP is not released. There are other molecules that are required for ATP release in ICD and are activated in a caspase-dependent rather than autophagy-dependent manner. They include lysosomal protein LAMP1, which is involved in lysosomal exocytosis and translocates to the cell membrane in ICD; pannexin 1 (PANX1) channels that release ATP from the cell; and Rho-associated, coiled-coil containing protein kinase 1 (ROCK1) that together with myosin-II is responsible for cell membrane blebbing [49]. In some cases of ICD, such as the one induced by hypericin-mediated photodynamic therapy, ATP release is autophagy-independent. Instead, it depends on PI3K-regulated exocytosis and PERK-mediated proximal secretory pathway [50].

The functions of eATP are mediated by two types of purinergic receptors - an ionotropic receptor P2Y2 (P2RY2) expressed in macrophages and metabotropic receptor P2X7 (P2RX7) primarily expressed in mastocytes, macrophages and DCs [51]. When eATP binds to P2Y2, it facilitates the recruitment of macrophages to the tumor microenvironment, i.e. acts as a "find-me" signal and promotes phagocytic clearance [52, 53]. The proinflammatory effects of eATP are mediated via the P2X7 receptor. Upon activation, it triggers the assembly of NLRP3 inflammasome in a CASP-dependent manner [54, 55]. This further induces the secretion of cytokines IL-1β and IL-18 that results in the priming of CD8+ T-cells, production of IL-17 by γδ T cells [56] and increased expression of IFN-γ [5].

In addition to the release by dying cells, ATP can be secreted by neutrophils to recruit other immune cells [51].

Extracellular ATP can be metabolized to ADP and AMP by CD39 (also known as ENTPD1 - ectonucleoside triphosphate diphosphohydrolase 1) and further to adenosine by CD73. These two markers can be expressed by tumor cells and stromal cells in the tumor microenvironment. In contrast to eATP, extracellular adenosine can act as an antagonist of eATP by suppressing the CD8+ T-lymphocytes. The binding of extracellular adenosine to purinergic receptors on the surface of CD8+ T-cells increases the intracellular levels of 3′,5′-cyclic AMP, thus activating immunosuppressive signaling pathways [57-60]. Therefore, the increased expression of CD39 and CD73 can reverse the proinflammatory effect of ATP release.

When eATP is enzymatically digested with apyrase or the production of ATP is inhibited, the immune cells' reactivity is significantly reduced [56]. Furthermore, loss-of-function polymorphisms of P2RX7 in breast cancer patients are associated with poor clinical outcomes [56]. These findings support the significance of ATP release in promoting immune response towards cancer cells.

Heat shock proteins in ICD. Besides the most studied DAMPs, many ICD inducers trigger the release of other molecules that are important for the immunogenicity of cell death. Heat shock proteins HSP70 and HSP90 are chaperone proteins from the ER, but they can be exposed on the cell membrane under stress conditions, including chemotherapy [13]. HSP90 externalization on cancer cells increases immunogenicity by promoting IFN-γ secretion by CTLs [5]. HSP70 exposure facilitates DCs maturation and activation of both CD8+ and CD4+ T-lymphocytes [1]. However, some studies showed very limited involvement of HSPs in immunogenicity or even immunosuppressive properties of these chaperones [5].

Experimental models for ICD studies

When comparing anticancer drugs with and without ICD-inducing properties, two key differences can be observed using *in vivo* animal models. Firstly, dying tumor cells treated with ICD inducer *in vitro* can act as vaccines. When cells treated with ICD inducer are administered to immunocompetent animals, this results in a protective immune response. When the vaccinated animals are inoculated with viable tumor cells of the same line, an adaptive antitumor response is triggered (Fig. 2, *a*). Such response can lead to the slower progression of tumor growth or it can even eradicate the inoculated malignant cells and prevent the growth of the tumor [61-63]. It is worth mentioning that the cells succumbing to ICD lead to the immune response without any additional adjuvants. Secondly, the efficacy of ICD-inducing drugs depends on the state of the host's immune system. ICD-inducing chemotherapeutics provide a more prominent antitumor response in immunocompetent animals while in the immunodeficient hosts, only a suboptimal response is detected (Fig. 2, *b*). For non-ICD-inducing compounds, there is no such difference in the response [46, 64, 65].

These two characteristics of the ICD-inducing drugs comprise the basis of the operational definition of ICD formulated by Kroemer et al. [23]. According to this definition, ICD must satisfy two criteria: first-

ly, dying cancer cells treated *in vitro* must induce a protective immune response in the host against re-inoculation of viable cancer cells of the same type; secondly, ICD induced *in vivo* must recruit innate and adaptive immune cells and elicit local antitumor immune response in immunocompetent hosts.

Since the action mechanism of ICD-inducing drugs requires the immune system of the host and depends on it, *in vivo* models are required to verify the immunogenicity of tumor cell death. While molecular markers of ICD can be detected using *in vitro* methods, *in vivo* studies are needed to confirm the immunogenic properties of the potential ICD-inducing chemotherapeutics.

The most commonly used tumor cell lines for ICD studies are the ones that form solid tumors in mice, e.g. murine lymphoma B16F10 and murine colorectal carcinoma CT26 [66]. However, here we demonstrated that ascites tumors like Nemeth-Kellner murine lymphoma (NK/Ly) might possess some advantages over them, such as faster progression and a positive correlation between animal body mass and tumor progression that makes the monitoring process easier. NK/Ly lymphoma has been used pre-

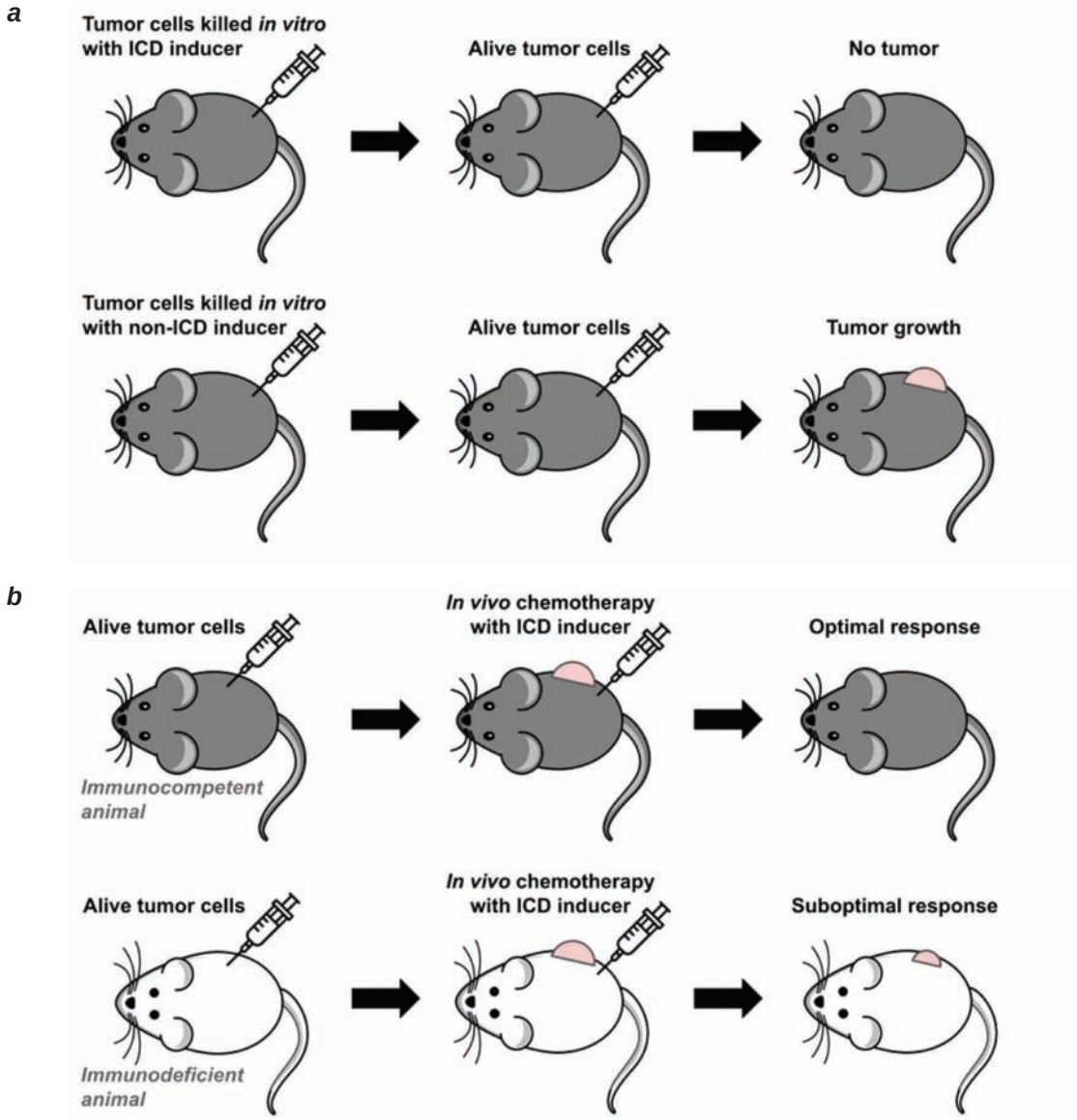

Fig. 2. ***a*** *– Dying cancer cells treated with ICD-inducing drugs in vitro act as vaccines in contrast to the cells treated with non-ICD-inducing drugs;* ***b*** *– the response to ICD-inducing chemotherapeutics depends on the state of the immune system: immunodeficient animals are significantly less responsive to therapy than the immunocompetent ones. Adapted from [23]*

viously in anticancer drug studies [67], but it has not been utilized specifically for ICD studies. In the current study, we propose the usage of NK/Ly lymphoma as a novel tumor model for ICD studies *in vivo*. We provide the results of its approbation for ICD-inducing chemotherapeutics doxorubicin (Dox) and oxaliplatin (Oxp), as well as for evaluation of the potential ICD-inducing capabilities of sanguinarine (Sang).

Materials and Methods

In vivo immunization studies were performed on C57BL/6 female mice kept under controlled temperature and 12-hout light/12-hour dark cycle on a standard diet (LLC "Vita", Obukhiv, Ukraine) and water supplied ad libitum. Nemeth-Kellner murine lymphoma (NK/Ly) cells used in this study were obtained from the collection of the Institute of Experimental Pathology, Oncology and Radiobiology (Kyiv, Ukraine) and cultivated *in vivo* for 14 years in the Institute of Cell Biology (Lviv, Ukraine). The ascites form of NK/Ly lymphoma was passaged by intraperitoneal inoculation in C57BL/6 mice. NK/Ly cells were obtained from the ascites drained on the 10-14th day from the abdominal cavity of anesthetized tumor-bearing mice.

All manipulations with animals were carried out following the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (1998), the Law of Ukraine "On the Protection of Animals from Cruelty" (2014), and approved by the Bioethics Commission of the Institute of Cell Biology NAS of Ukraine (protocol 1-2024).

NK/Ly cells were seeded in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (Biowest, France) and treated for 24 h with doxorubicin, oxaliplatin and sanguinarine to determine cytotoxicity and IC_{50}. Cells were cultivated in a humidified incubator at 37°C in an atmosphere of 5% CO_2. Concentrations of 5, 10 μM of Dox, 50 μM of Oxp and 10, 25 μM of Sang manifested the most prominent cytostatic and cytotoxic effects and were selected for further immunization studies *in vivo*. NK/Ly cells treated with drugs in these concentrations were used to inoculate C57BL/6 female mice ($5{\cdot}10^5$ cells per animal). Intact and frozen (4 h at -70°C) NK/Ly cells were used as positive and negative controls, respectively. Animals were then reinoculated with intact NK/Ly cells ($5{\cdot}10^5$ cells per animal) 14 days after initial inoculation. Blood samples were collected from surviving animals 2 days before and 30 days after the reinoculation and analyzed on an automatic blood analyzer Dymind DF51 Vet.

Results and Discussion

NK/Ly lymphoma was found to be internally resistant to all studied chemotherapeutic compounds *in vitro*. In particular, the IC_{50} of Dox was 1.4 μM, while the IC_{50} of Oxp and Sang were 28.5 and 7.9 μM, respectively (Fig. 3), which is higher than the IC_{50} for other cell lines (e.g. Jurkat T-leukemia cells). This may indicate overexpression of ABC transporter proteins in NK/Ly lymphoma cells, and further clarification is needed.

Both Dox and Oxp, the well-established ICD-inducers [6], demonstrated some degree of efficacy in NK/Ly tumor immunization studies (Fig. 4). In particular, 50% of animals which were injected by Dox-treated NK/Ly lymphoma cells proved to be immune to reinoculation by alive tumor cells. In comparison, for Oxp, this index was significantly lower - only 12.5% - 25% in different experiments. Tumor-free survival and overall survival rates in the immunized mice were significantly longer compared to controls. On the contrary, all animals inoculated by freeze-thaw control (i.e. necrotic tumor cells that were killed without inducing ICD) did not observe

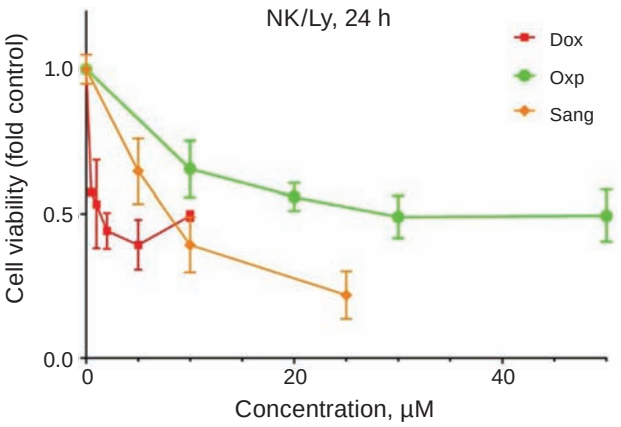

Fig. 3. NK/Ly cells were cultivated in vitro and treated with Dox (0.5, 1, 2, 5, 10 μM), Oxp (10, 20, 30, 50 μM) and Sang (5, 10, 25, 50 μM) for 24 h to determine toxicity and IC_{50}. Cell viability was assessed using trypan blue assay. The graph shows relative counts of viable cells. IC_{50} values: Dox: 1.4 μM, Oxp: 28.5, Sang: 7.9 μM. IC_{50} was calculated using GraphPad Prism 9. Dox – doxorubicin; Oxp – oxaliplatin; Sang – sanguinarine

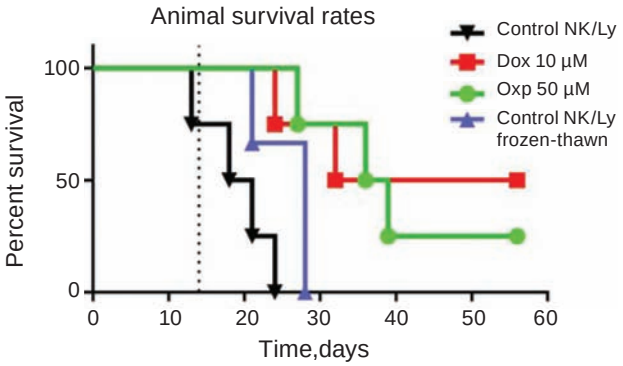

Fig. 4. Animals were inoculated with dying NK/Ly cells treated with Dox (10 μM) and Oxp (50 μM). Viable and frozen-thawed NK/Ly cells were used as controls. After 14 days (shown as a dotted line), animals were rechallenged with viable Nk/Ly cells. Animals immunized with Dox- and Oxp-treated NK/Ly cells have demonstrated more prolonged survival after the rechallenge. Each group contained 4 animals. Dox – doxorubicin; Oxp – oxaliplatin

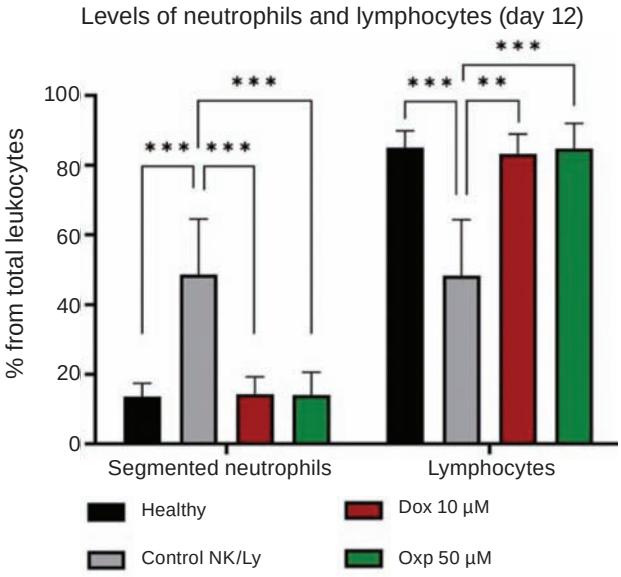

*Fig. 5. Analysis of blood formula of tumor-bearing C57BL/6 female mice. Compared to the healthy group, the animals that developed ascites tumor after inoculation with untreated NK/Ly cells demonstrated higher neutrophils to lymphocytes ratio. In contrast, animals immunized with doxorubicin- and oxaliplatin-treated NK/Ly cells did not show significant differences in neutrophils and lymphocyte levels compared to healthy animals. **$P \leq 0.01$; ***$P \leq 0.001$ (pairwise comparisons). Each group contained 4 animals. Statistical analysis was performed in GraphPad Prism 9. Dox – doxorubicin; Oxp – oxaliplatin*

any signs of immunity towards NK/Ly lymphoma. The observed immunization effects correspond to the operational definition of ICD (Kroemer, 2013) and suggest that ICD may occur in NK/Ly lymphoma treated by Dox and Oxp. Further studies of DAMPs release by treated NK/Ly cells can provide more evidence for that. Sanguinarine, despite high cytotoxic activity *in vitro* (IC_{50} = 7.9 μM), has not demonstrated effectiveness in immunization studies.

Immunization of Dox- and Oxp-treated animals was also accompanied by normalization of the number of total lymphocytes and neutrophils compared to the control group (Fig. 5). This evidence suggests that the immune system may play a crucial role in Dox- and Oxp-induced cell death in NK/Ly lymphoma *in vivo*. To confirm this hypothesis, further studies of lymphocyte subpopulations are needed.

Conclusion. We have shown that NK/Ly lymphoma treated by doxorubicin or oxaliplatin is effective in the immunization of immunocompetent mice, which corresponds to the operational definition of immunogenic cell death. This suggests that ICD takes place not only in solid but also in ascites tumors. Therefore, NK/Ly lymphoma is a suitable candidate for further studies of ICD-specific DAMPs release and a promising model for ICD induction studies both *in vitro* and *in vivo*.

Conflict of interest. The authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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БІОХІМІЧНІ ТА КЛІТИННІ МЕХАНІЗМИ ІМУНОГЕННОЇ ЗАГИБЕЛІ КЛІТИН

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Імуногенна загибель клітин (ІЗК) – це режим запрограмованої клітинної загибелі, що призводить до активації протипухлинної імунної відповіді та визначає довгостроковий успіх протиракової терапії. Тут ми надаємо огляд відомих молекулярних і клітинних механізмів ІЗК. Зазвичай у дослідженнях ІЗК використовуються експериментальні моделі солідних пухлин. Проте моделі асцитних пухлин можуть мати деякі переваги перед ними. У статті наведено результати нашого дослідження з апробації мишачої лімфоми Немет-Келнера як експериментальної моделі асцитних пухлин для досліджень ІЗК.

Ключові слова: імуногенна загибель клітин, біохімічні механізми, модель асцитних пухлин, мишача лімфома Немет-Келнера, доксорубіцин, оксаліплатин.

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