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Activating Effect of Staphylococcal Enterotoxins

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Abstract

The effect of enterotoxins is to induce the production of endogenous IF. *St. aureus* enteropathogenic proteins (enterotoxins) possess an antitumour effect. After intraperitoneal inoculation, they decrease the size and, in some cases, prevent the development of the human hypernephroma in the cheek pouch of golden hamsters. The effect of enteropathgenic proteims may possibly consist in inducing the production of endogenous immune interferon which activates the host immune system and enhances the rejection of heterologous tumour cells.

Keywords

Gamma-Interferon, Enterotoxins, Antitumour Activity

1. Introduction

Staphylococcal enterotoxins (SE) were first described and designated serotypes A, B, C by Bergdoll [1]. Unlike enterotoxins, which have a subunit structure, the protein of staphylococcal enterotoxins is a single-chain consisting of 22 - 24 amino acids. The sequence connected by a disulfide bond. Molecular weight of SE proteins no more than 20 - 25 kD.

Being the main factor of enteropathogenicity, staphylococcal thermostable enteropathogenic proteins also have superantigenic activity and the ability to confuse antibiotic resistance [2].

The *St. aureus* enteretoxins function is both as potent gastrointestinal toxins as well as superantigens that stimulate non-specific T-cell proliferation.

In the enterotoxin molecule, the sites that control the secretion of the main factor of enteropathogenicity and superantigenic activity have different domain localization [2].

The impact of enterotoxins on the immune system is carried out through superantigenic activity, determined by the corresponding domain structure of the molecule.

Staphylococcal enterotoxins possess low antigenic activity and low toxicity in animal experiments. The exception is type B (SEB), which has high lethal activity [3].

This article presents the results of the immunomodulatory effect of SEA on human splenocytes, the secretion of interferon gamma and the antitumor effect in animals.

2. Experimental Part

In the experiments, preparations of staphylococcal enterotoxins were used, previously obtained by the ion exchange cellulose method [4]

2.1. Binding of Staphylococcal Enterotoxin A (SEA) with Human Splenic Lymphocytes, 1983 [5]

Summary:

1) In experiments studying binding to human splenocytes, staphylococcal enterotoxin thia A, labeled [I125] SEA, was used. A high degree of specific binding of [I125] SEA to human splenocytes has been established.

2) It has been established that the binding of SEA to the surface of splenocytes at 4 C is characterized by saturation, reversibility and high affinity for the receptor Kd = 4.0×10^{-7} M).

3) The number of binding sites on a splenocyte cell is 60,000.

4) The effect of temperature on the binding of SEA to the surface of the splenocte was studied. It was shown that at 23˚C, the binding of the labeled toxin to the cell is described by a two-phase curve.

5) The conditions under which there is an increase in the connection of SEA with splenocytes and, accordingly, an increase in the level of interferon gamma production, have been determined.

Printing increases the association of SEA with the splenocytes and correspondently increases the production level of gamma-interferon.

2.2. Nonspecific Antitumour Activity of Staphylococcal Enterotoxins, 1987 [6]

It is known that the components of the interferon (IF) antitumour activity include the direct effect on tumour cells and the indirect effect on various body systems [7] [8]. It is not entirely clear what is the contribution of each IF effect to the tumour process inhibition. Thus, in thymus-deprived mice, a slowdown in the growth of transplanted human tumours was noted when they were injected with both human and mouse IF [1]. In addition, there are reports of the inhibitory effect of IF on tumour development *in vivo* with the resistance of tumour cells to IF *in vitro* [9].

Inductors of IF are also used in the experiment for the tumour treatment [10].

Staphylococcus aureus proteins deserve special attention in this regard—staphylococcal enterotoxins, which are also active immunomodulators [11] [12]. Immune IF produced by T-lymphocytes induced by these proteins has a pronounced antiproliferative effect [13].

We studied the effect of *St. aureus* enterotoxins on the development of human hypernephroma in the cheek pouch of golden hamsters.

2.2.1. Methods

The passaged culture of human hypernephroma was obtained from cells of a similar tumour. The culture has passed more than 200 times; it contains specific markers and has properties inherent to the primary tumour [14]. The culture was passaged on the Eagle medium with lactalbumin hydrolysate (1:1) and 10% bovine serum. The culture formed a monolayer with growth, the passage index was 1:3. As an experimental model, 50 golden hamsters weighing 250 g were used supplied by the kennel Stolbovaia of the USSR Academy of Medical Sciences, to which, 4×10^6 cells of human hypernephroma were injected into the cheek pouch under Nembutal anaesthesia.

Human leukocyte IF (HLI) was obtained by the method described by V. D. Soloviov, T. A. Bektemirov [15], human immune IF (HII)—according to the method proposed by L. M. Mentkevich *et al.* [16]. The potency of HLI and HII was 1000 U/mL. Enterotoxins A and B of St. aureus were obtained by the method of Ezepchuk *et al.* [4]. IF and enterotoxins were injected into golden hamsters intramuscularly or into the abdominal cavity in a volume of 1 mL daily, starting from the next day after human hypernephroma cells administration. The size of the tumours was considered 2 weeks after the experiment start. The level of IF in the blood serum of golden hamsters was determined by titration on human embryo fibroblast cultures and the transplanted culture of BHK-21 golden hamster. Titration was carried out by micro method on a monolayer of cells inoculated into 96-well plates. The IF titre was considered as its last dilution protecting cells from the cytopathic effect of 100 doses of the vesicular stomatitis virus. The titre of antibodies to staphylococcal enterotoxin in the blood serum of golden hamsters was determined 3 weeks after the start of experiments by immunoprecipitation.

2.2.2. Results and Discussion

A preliminary study of HLI showed that it had absolute species specificity: it showed antiviral effect in the culture of human embryo fibroblasts and had no effect on the culture of BHK-21 golden hamster. The culture of human hypernephroma cells turned out to be weakly sensitive to the antiviral effect of HLI, as well as other transplanted human cultures, and sensitive to its anti-proliferative effect. On this basis, it was concluded that human hypernephroma cells injected into the cheek pouch of golden hamsters should be sensitive to the virus injected into hamsters, and due to its species specificity, it should not affect the host organism.

Human hypernephroma cells were injected into the cheek pouch of 30 golden hamsters. Then, the animals were divided into five groups. Animals of group I, which were not injected with IF, were the control; HLI was injected into the abdominal cavity of group II animals; intramuscularly into group III animals; HII was injected into the abdominal cavity of group IV animals; intramuscularly into group V animals.

In animals of groups II and III, compared with animals of group I, neither the incidence of tumours nor their size decreased (**Table 1**). The introduction of HII to golden hamsters had a therapeutic effect. So, tumours occurred in 50% of group IV animals compared to 83.3% in the control. The average diameter of tumours in group IV animals was significantly lower than in control animals: 1.0 ± 0.57 and 4.0 ± 0.93 mm, respectively, the difference is significant. In golden hamsters of group V, the incidence of tumours was the same as in the control, and the tumour size was lower (2.0 ± 0.57 mm). However, due to the large spread of figures, these differences turned out to be not significant. So, the HII injected into the abdominal cavity prevented the occurrence, and in case of occurrence, inhibited the development of human hypernephroma in the cheek pouch of golden hamsters.

One of the differences between the used drugs of IF was that HLI did not contain an active inducer of IF, whereas HII contained staphylococcal enterotoxin A (SEA). We suggested that the therapeutic effect of HII could be associated not only with the administered IF, but also with SEA, which could induce the production of endogenous immune IF in the body of golden hamsters. To test this assumption, intact hamsters were injected with native HII into the abdominal cavity and then blood was sampled over time. The presence of IF in the blood serum of golden hamsters was determined in the monolayer of human fibroblast cells and hamster cells.

HII in the blood was not detected throughout the experiment. Apparently, it was quickly adsorbed by tissues and excreted from the body. IF of hamsters was detected in the blood of animals 15 minutes after administration of the IF drug reaching a maximum after 1 h (64 U/mL) and disappearing after 6 hours from the bloodstream. Therefore, SEA contained in native HII induced the production of endogenous homologous IF in the body of golden hamsters.

Several types of enterotoxins were isolated from *St. aureus*. Enterotoxins A and B have the same interferon-inducing, but different immunomodulatory property [17].

Native HII into the abdominal cavity: 1-IF titration on the VNK-21 culture, 2-IF titration on the human fibroblast culture.

The objective of this series of experiments was to study the antitumour effect of enterotoxin B (SEB), since it is less toxic than SEA. Otherwise, the experiments were planned similarly to those already described. From the next day after the introduction of human hypernephroma cells, golden hamsters were injected daily into the abdominal cavity with 1 or 0.1 μg of SEB. 2 weeks after the administration of a higher dose of SEB, tumours in the cheek pouch appeared in

16.6% of golden hamsters compared to 85.7% in the control. The mean diameter of tumours in experimental animals was 0.66 ± 0.67 mm, and in control animals was 3.6 ± 0.61 mm ($P < 0.01$). When a lower dose of SEB was administered, the number of experimental animals with developed tumours was the same (83.3%) as in the control (85.7%), the average diameter of tumours in experimental animals was slightly lower than in control animals (2.65 ± 0.62 mm), but the difference was not significant (Table 2).

Thus, SEA and SEB slow down and in some cases prevent the development of human hypernephroma in the cheek pouch of golden hamsters. The effect of enterotoxins is to induce the production of endogenous immune IF, which activates the body's immune system and enhances the effect of heterologous tumour cells rejection noted in the model system used. We cannot exclude the direct stimulating effect of enterotoxins on the immune system of golden hamsters.

St. aureus enterotoxins are proteins with weak antigenic properties. In our experiments, after repeated administration of these proteins, antistaphnococcal antibodies in titres 1:2 - 1:4 were detected in the blood of golden hamsters with implanted human hypernephroma cells. With the same scheme of enterotoxins administration to intact hamsters, the level of antibodies was significantly higher (1:16). It has been established that even a small amount of anti-staphylococcal antibodies in the blood can serve as an obstacle to the enterotoxin action. In this regard, for further work, it is necessary to obtain fragments of proteins that do not cause the production of antibodies, but have an antitumour effect.

2.3. Effect of Spleen Cells Sensitized with Staphylococcal Enterotoxin Type A on Metastasis of Lewis Lung Carcinoma in Mice, 1989 [18]

The effect of intact murine splenocyte cells sensitized *in vitro* with staphylocoeic enterotoxin (SEA) on spriading of mmouse Levis carcinoma was studied.

A significant reduction in the number of lung metastases and lung weight was found after multiple intrapulmonary inoculation of splent cells treated with SEA for 6 hours. The effect was less noticeable after inoculation of sensitized cells intraperitoneally or sensitized intraperitonialy or into the femoral muscle of the leg affected with the tumor.

Table 1. The development of tumours in golden hamsters after the introduction of human hypernephroma cells exposed by HLI and HII.

Introduced IF	Administration method	Tumour incidence, %	Tumour diameter, mm (M ± m)
HLI	Abdominally	83.3	3.4 ± 1.16
HLI	Intramuscularly	83.3	4.6 ± 1.54
HII	Abdominally	50.0	1.0 ± 0.57*
HII	Intramuscularly	83.3	2.0 ± 0.57
Control	–	83.3	4.0 ± 0.93

*The difference is significant ($P < 0.05$).

Table 2. The development of tumours in golden hamsters after the introduction of human hypernephroma cells exposed by SEB.

Experiment settings	Drug concentration, μg	Tumour incidence, %	Tumour diameter, (M ± m)
Test	0.1	83.3	2.65 ± 0.62
	1.0	16.6	0.66 ± 0.67*
Control	–	85.7	3.6 ± 0.61

*The difference is significant ($P < 0.01$).

3. Discussion and Conclusion

Staphylococcal superantigennenic enteropathogenic proteins, which have weak antigenic activity, exhibit high immunomodulatory properties in animal experiments. It has been shown that the intensity of gamma interferon secretion correlates with the presence of SEA binding compounds on the surface of splenocytes. The antitumor effect is apparently due to the effect of immune interferon on oncogenic cells [19].

The fact of different domain localization in the enterotoxic protein molecule indicates that only the superantigenic activity of the toxin is related to the secretion of immune interferon. Of great interest is the spatial structure of the domain that controls superantigenic activity. Deciphering the molecular structure of the domains holds the key to the use of enteropathogenic proteins for medical purposes [20].

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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