Interferon production

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Interferon

- Interferons are natural glycoproteins produced by virus-infected eukaryotic cells which protect host cells from virus infection.
- The substance was called interferon because it interfered with intra-cellular multiplication of viruses.
- Viral interference is a phenomenon observed when multiplication of one virus is inhibited by another virus. For instance, when influenza-A virus is inoculated into the allantoic cavity of an embryonated egg followed after 24 hr by influenza-B virus, the multiplication of influenza-B virus is partly or completely inhibited. The reason why influenza-B virus cannot multiply is that the influenza-A virus infected cells produce interferon which partly or totally inhibits multiplication of B virus. The interferon also protects cells from influenza A virus.

History

- They were discovered by Isaacs and Lindenmann in 1957 in course of a study of the effect of UV-inactivated influenza virus on chick chorioallantoic membrane kept in an artificial medium.
- They observed that the infected membrane produced a soluble substance in the medium which could inhibit the multiplication of active influenza virus inoculated in fresh chick chorioallantoic membranes.

Characteristics of Interferons

- An outstanding feature of interferons is that they are host-cell-specific and not virus-specific.
- This means that interferons produced by mouse or chicken will not protect human cells against the same virus which induced interferon in the experimental animals.
- On the other hand, an interferon produced by a virus X in an animal will protect the animal also from other viruses.
- The reason why interferon produced by one species does not protect another species is that the same virus produces different interferons in different species.

Types of human interferons

- Human interferons are of three main types:
  - alpha interferons (α-IFN): Alpha-interferon contains many subtypes. The total subtypes exceed 20 in number. It is produced by the B-lymphocytes, monocytes and macrophages.
  - beta-interferons (β-IFN): β-IFN is produced by the fibroblasts in the connective tissues.
  - gamma-interferons (γ-IFN): γ-IFN is synthesized by the T-lymphocytes after they are activated by antigens.

Production of interferon

- Interferon is produced by growing a continuous cell line in a suspension culture.
- The cells first being primed by cultivation in the presence of interferon specific to the cells and L-glutamine.
- Interferon production being initiated by contacting the cells with a high concentrated suspension of virus inducer, then removing the virus and incubating the cells in a nutrient medium.

Production Steps (taking example-human specific interferon prepared from "Namalva" Burkitt cell line and virus inducer “Newcastle disease virus”)

This line was isolated by Prof. George Klein of the Karolinska Institute in Stockholm from an African suffering from Burkitt's disease. It was selected by Dr. Hans Strander of the same Institute for its high capability for producing interferon.

- A process for the preparation of interferon of high titre which comprises growing interferon-producing cells from a continuous cell line in a stirred suspension culture while effecting the following process stages:
- "Namalva" cells were sown at a concentration of 2×10^5 cells per ml in a RPMI 1640 nutrient medium supplemented by 10% of calf foetus serum. The cells were cultivated in suspension in a 20-liter tank at 37°C, with mechanical stirring (50 rpm, At too high a stirring speed, the cells would be killed by mechanical shearing, and if too slow, the culture would be insufficiently oxygenated), until the cell saturation density was obtained. This was 2.5×10^6 cells/ml. This saturation density was reached after growth for 72 hours.

***Immortal cell cultures are called continuous cell lines, to distinguish them from primary cultures and cell strains which have definite lifespan. Normal cells usually divide only a limited number of times before losing their ability to proliferate, which is a genetically determined event.**

...Production steps

- **A priming amount of 10 units of human interferon per ml and 0.5 mg/ml of L-glutamine to keep the cells in an active metabolic state were then added. Priming was continued for 18 hours, at 37°C.**
- **During this stage, the cells were allowed to deposit under gravity, and after 18 hours the supernatant liquid was sucked off.**
- **The residual medium containing the cells was centrifuged, and the resulting cell mass was resuspended in 0.01 of the original culture volume of a suspension of virus inducer (live virus, Herz strain, of Newcastle disease) to provide a multiplicity of infection of 1 (for about 1hour), and thereafter removing the virus inducer by centrifugation (reused more than 8 times)**

***Immune cells largely depend on glutamine availability to survive, proliferate, and function, and ultimately defend our body against pathogens.**

****Interferons can prime cells, increase their sensitivities to viral genome toxicity, inhibit cell multiplication, and induce a variety of immune modulations. The priming effect of interferon, itself, appears to consist of several cellular alterations. The priming effect of interferon pretreatment on interferon production can also shorten the lag period between addition of inducer and the appearance of interferon in induced cultures.**

... Production steps

- The Burkitt cells were resuspended at a concentration of 10^7 cells/ml in RPMI 1640 medium, which had been supplemented by 2% of calf foetus serum and contained 10 micrograms per ml of cycloheximide (metabolic inhibitor).
- After several hours exposure to the cycloheximide, the latter was removed by centrifugation, and the cell mass was resuspended in RPMI 1640 nutrient medium, without serum, at a concentration of 10^7 cells/ml, and incubated for 18 hours with stirring.
- The resulting cells were allowed to deposit under gravity, and the supernatant liquid containing the interferon was sucked off, concentrated, and purified by known methods.

***Cycloheximide, a specific inhibitor of cytoplasmic protein synthesis (added so that cell use its full energy for mitochondrial activity). Mitochondria have a central position in innate immune response via the adaptor protein MAVS in mitochondrial outer membrane to limit viral replication by inducing interferon production**

Suspension culture vs monolayer culture for interferon production

- Interferon can be produced by the growth of animal cells in monolayer cultures, and this can be used to produce interferon preparations of high titre.
  - However, monolayer culture techniques do not lend themselves readily to industrial-scale production.
  - They have an inherent limitation since the cells grow on a solid surface, and in order to obtain an adequate oxygenation rate for cell growth, a high ratio of cell surface to medium volume is required.
  - This culture technique also involves a large number of operations requiring very specialised handling, each handling operation involving a risk of contamination.
- Animal cells can, however, be grown in virtually unlimited quantities, in stirred suspension culture in a single vessel. Oxygenation, pH, and the introduction of nutrients can be automatically controlled to obtain increased cell populations, and a more efficient utilisation of the nutrient medium, than in the case of monolayer cultures.

Questions

- What are interferons? Write an essay on industrial production of interferons.
- Write various steps of interferon production
- Suspension culture vs monolayer culture method for interferon production.