

Chapter 2

Immunological Defence Mechanisms Against Biological Agents

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Abstract A short discussion about attack strategies by microorganisms and immunological defence mechanisms by human body: facts and concepts for preventing disease and biological warfare.

Keywords Immunology • Microbes • Prevention • Vaccines

2.1 Immunology, How It Works

Immunology is a rather young science studying biological mechanisms existing in biological evolution since the earliest multicellular forms of life (sponges) and becoming progressively more complex in mollusks, insects, fish, birds up to mammals and humans. Immunology is a little more than 50 years old, with this specific name, although the first informations on the existence and validity of immune defence go back indeed to Edward Jenner with his “vaccination” practices in the 1790s.

That first “immunology” experiment is something done a little more than two centuries ago that is not ethically feasible today [1] ...

Jenner, noticing that english milkmaids who caught cowpox did not get human smallpox, voluntarily and deliberately exposed his gardener’s son (James Phipps) to cowpox (causing a fever illness); after recovery he challenged him with smallpox verifying his attained “immunity”.

Jenner is therefore considered the father of “immunology”. No mechanistic explanations of that experiment were possible at that time: only about 100–150 years later we started discovering antibodies and immune system functions allowing us to

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understand what was biologically happening in the milkmaids and in the “vaccinated” individuals like Jenner’s gardener’s son.

The immune system is in fact like an “eye within” the body, vigilating that nothing extraneous is biologically active in each individual organism, recognizing effectively “self” from “not self”, ensuring that replication of self cells is not contaminated by “foreign” biological agents and killing any extraneous biological entity through the action of soluble “light” weapons (antibodies) and of “heavy” killers (immune cells and macrophages, interacting) [1].

The key steps of an immune reaction are substantially three: (a) foreign particles inglobated by macrophages are degraded and “presented” to T-Helper lymphocytes which in turn start an elaborated attack, mostly through either (b) soluble weapons (antibodies, humoral response) or (c) cellular weapons (killer T-lymphocytes, cellular or cytotoxic response) or both (b+c), depending on the case.

Recently, it has been shown that activated T-lymphocytes tend to aggregate, like a swarm of bees, exchanging informations, useful to coordinate the immune response (i.e. to elaborate coordinated defence plans) [1].

When invaders are present in body fluids (extracellular, like most bacteria) they can be attacked with antibodies (specific surface recognition).

When invaders are instead intracellular (viruses, some bacteria like TBC) they are attacked with special killer cells (cytotoxic T-Ly) destroying self cells harboring the intruders, with their content [1].

Viruses have also a transit in body fluids, so antibodies are also produced against them.

In most responses, both humoral (antibodies) and cellular attacks are actuated. The immune response, moreover, is specific and potentiated by memory: therefore a second or further encounter with the same foreign agent (antigen) produces a stronger and quicker defense reaction [1].

2.2 Microbes, How They Prevail (Attack Strategies)

If germs make it to penetrate in the body, they enter an hostile territory, where they try to survive through strategic actions: rapid proliferation, identity disguise, toxic products (toxins), resistance to attacks, niche hiding, etc.

If microbes prevail, they can multiply and pass to other individuals.

The capacity to hide from immune attacks is exemplified by *Listeria monocytogenes*, which is able to escape vacuoles when internalized. The bacterium so hides and passes from cell to cell by endocytosis going undetected by antibodies.

Also tuberculosis (TBC) represents a great challenge, because the organisms (*Mycobacterium tuberculosis*) can survive even after they have been internalized (phagocytosed) by macrophages. The mycobacteria can indeed hide inside the bone marrow stem cells and evade the immune reactions for many years [1].

But fortunately, highly pathogenic germs are very few.

Millions of species of microorganisms exist. Most of them are not pathogenic for humans, because the immune system easily destroys them. The very limited number of effective pathogens is characterized by special properties making them resistant to the immune attacks.

Very virulent germs tend to disappear in nature, because they kill their sensitive hosts (natural selection): the killer disappears together with the killed. However, many virulent germs tend in time to attenuate their aggressiveness (which might make them extinguish), allowing milder forms of infection to emerge, that do not kill the hosts and guarantee survival of both contenders (parasite and host).

The attack strategies by germs are mostly implemented by rapid proliferation, which is a key factor for microorganisms: immune response indeed requires some time (7–10 days) to be strong. Another feature of attack by germs is their capacity to elaborate toxins (blocking key functions of the host): movement (tetanus), water balance (cholera), etc.

Further strategies are immune evasion (antigenic change – flu viruses), complement inhibition, resistance to phagocytosis, or even T-Ly elimination (HIV) [1].

2.3 Prevention to Favour Defence and Immunity Success

To control infections and favour immune responses, we may try:

1. to reduce microorganism proliferation (through the use of bacteriostatic drugs) or kill the germs (disinfection, use of bactericidal drugs);
2. to control the vectors diffusing the infection (insects, arthropods, birds, rats, etc.);
3. to immunize preventively the potential hosts (vaccinations): this requires public health planning, technology, costs and time;
4. to administer preformed antibodies (serotherapy): this also requires technology, costs and time.

Actions against germs (point 1, above) implies the use of chemotherapeutic agents (antibiotics) having a multitude of action mechanisms and being suitable under precise conditions; germs tend to develop resistance to them; therefore, fewer and fewer effective antibiotics are available today, also because the discovery of new molecules in this category has been scarce, lately. Hygienic measures are very effective, well known and can be enacted at low cost, whenever possible.

Campaigns against vectors (insecticides, biological competitors – fungi, bats, genetics -) can also be very effective (if they are done with a good biological knowledge of the ecological system) [1, 2].

But the best possible actions are the use of vaccines (if available and if time allows) and of immune sera (if available).

A vaccine is always preferable, because induces an active response and an advantageous state of immune memory in the individual, with minimal side effects (active immunization). A serum specific for a given antigen can be life-saving (serotherapy,

immediately active), but has some side effects (since it introduces heterologous proteins -for instance, horse immunoglobulins, which in turn will be immunologically eliminated-) and does not have a lasting protection (passive immunization).

Modern life has created some extra occasions for germs and some “new” diseases [2]: air conditioning apparatuses for instance (if not properly cared and cleaned) are a new ideal environment for bacteria; because of dirt and humidity they can foster growth of airborne bacteria never seen before as human pathogens: *Legionella pneumophyla*. The story of Legionaries disease (a fatal lung infection by *Legionella*) is very instructive for microbiologists and epidemiologists. Human behaviours (homosexual intercourses, exchange of syringes among intravenous drug users and frequency of air travel) have extended the contagion of formerly rare infections like HIV: also in this case the facts are dramatically instructive for medicine and epidemiology. Transfusions of unscreened blood have also diffused hepatitis and HIV viruses. Centralized processing of foods has sometimes diffused a contagion of food-borne infections (*E. coli*) [1].

Airplanes have replaced ships for human travel and are at the center of attention for human communicable diseases spreading (Influenza, SARS, etc.). Many more people are traveling today to remote and tropical areas than before (forests, wilderness): this can expose more populations (even at home, on the return) to rare insects and microbes (see cases of malaria, Ebola virus, Marburg virus, etc.). In addition, economic development expands contacts: mining, forestry, agriculture in new tropical areas with recent deforestation.

And more, the increasing number of subjects with immunodeficiency diseases or immunosuppressant therapies (for transplants) increases the probability of new communicable agents to infect people, survive, and propagate in modern societies (opportunistic infections, with possible mutations in progress).

This entire panorama increases the variety of new emerging infections: here is a variety of bacterial and viral pathogens of recent discovery: Rotavirus, *Cryptosporidium parvum*, *Legionella pneumophyla*, Ebola virus, Hantaan virus, *Campylobacter jejuni*, HTLV virus, HIV virus, *Helicobacter pylori*, Herpesvirus-6 and -8, virus Guanarito, virus Sabia, nCoV-MERS virus, to name just some of the most relevant and recent [2].

So, we have a lot to learn about new infections and a lot to do to control communicable diseases, also because of the continuously changing human ecosystem, in consequence of the ever changing human activities and the rapidity of genetic changes taking place in many microorganisms.

2.4 Vaccines: How to Prepare Them

What is a vaccine? In a dangerous disease caused by microorganisms and “foreign” not-self substances (an “antigen”), it may be possible to raise a vaccine, that is an innocuous preparation of that “antigen” which can induce the production of an immune response and create immune memory in the subjects we want to protect.

A vaccine can be developed in many ways: killed (denatured) or non replicating pathogens (viruses), recombinant protein antigens, live, attenuated (less harmful) strains of pathogens (for instance cowpox virus to protect against smallpox ...)

Vaccines can be very effective: cases of smallpox (extinction of the disease: non more cases of smallpox exist on earth for the success of global vaccine campaigns) and of polyo (near extinction of disease, except in some nomad populations and in critical areas of today's world, like Syria and also, surprisingly, of remote China provinces) are very instructive on the subject [2].

Requirements for vaccine preparation are the following. (a) An innocuous preparation of the antigen (+/- adjuvants); (b) a suitable biologic model in which to test the effectiveness of the immunization procedure; (c) a reduced series of administrations able to induce a long lasting response (number of boosters required): polio (very long lasting), as opposed to tetanus (lasts only some years, many boosters required); (d) an easy and affordable procedure for preparing quickly a large amount of product to use in case of necessity [2].

With such common and general requirements, the most used vaccinations include

1. killed whole germ preparations (influenza; polio type Salk; Hepatitis A; rabies; pertussis; cholera);
2. live attenuated germ preparations (tuberculosis; mumps-parotitis-rubella; polio type Sabin; yellow fever; variola; typhus and lately also against Ebola and Marburg viruses);
3. purified component vaccines (meningitis – subunits of *Haemophilus influenzae* b; acellular pertussis vaccine; tetanus-diphtheria anatoxins);
4. recombinant DNA vaccines (surface antigen of hepatitis B);
5. naked DNA vaccines (against many different germs, not yet widely used);
6. vaccines in genetically modified plants (against many different germs, not yet widely used) [2].

Technology of vaccine preparations is continuously evolving, in order to improve our capacity to prepare quickly enormous amounts of vaccinating doses from micro-organisms soon after their isolation in newly appearing forms or strains. For viruses, for instance, an important step forward has been the technology of reproducing the organisms on cell cultures rather than on fertilized chicken eggs.

2.5 Vaccination Campaigns: How Best Use Vaccines

(supplies/demands)

Mass vaccination campaigns have taken place several times in history, like those of 1947 against smallpox, in which the citizens of New York City stood in long lines to be vaccinated at a rate of eight persons per minute, and like those of several countries against polio (after the Sabin vaccine introduction, in 1960), with periodic

vaccination days organized to reach many secluded groups of citizens living in remote areas [2].

The realities however are that the existence of a vaccine is not enough to prevent a worldwide pandemic; more testing is needed before a new vaccine may be offered to the public; for vaccines prepared on chicken eggs, the successful mass production depends on the availability of the eggs, indeed; obstacles include organizational system for distribution and timely supply of doses, where and when they are needed.

Good news for scientists and for biologists is that since the September 2001 terroristic attacks in the USA (anthrax spores in the mailings), the employment needs of biologists have increased remarkably (production of vaccines, enactment of protection plans, treatment schemes for infections, etc.) [1, 2].

The field of immunology, in particular for vaccine research and development, continues to be of high impact in modern medicine and of high relevance in the contrast of bioterrorism.

To conclude, science is here to help; to be of use; to spread not infections but knowledge, across different cultures and nationalities.

Our role of scientists is mostly that of being “pontifices” (latin “pontes facientes”, that means “bridge makers” across cultures and nationalities). For this reason it has been a real pleasure to exchange here our knowledge and to discuss it together, among so many different people and so wide scientific expertises.

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