

Chapter 1

Immunorecognition of Biological Agents: An Introduction to Immunology

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Abstract The main mechanisms and concepts regulating the functioning of the human immune system are illustrated, with the general aims of improving disease prevention and ecosystem dynamics knowledge. These concepts must be known for the best management of infectious diseases and in the paramount view of ecology and parasitism, which are at the basis of stage-sharing of the environment by different species of life, each defending its own uniqueness and biological existence. Man is only one of the many living species sharing the stage of the planet. These considerations are fundamental in the fields of infectious diseases and of bioterrorism which have to be efficiently contrasted.

Keywords Immunology • Parasitism • Biological defense mechanisms • Bioterrorism

1.1 Introduction

The far reaching aims of this NATO-Advanced Study Institute course allowed us to delineate a very wide landscape of scientific perspectives. Therefore, for the teaching purposes set here, we are sharing some of our studies in general immunology and some scientific and philosophical considerations concerning biological agents living in the environment and their relations with humans, in the very wide endeavours of ecological relationships, parasitism, immunological defenses and infectious

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disease mechanisms. All these concepts must be studied not only for the biological survival and defense of each individual in the environment but also for social prevention purposes, in the event of criminal use of biological agents (bioterrorism) aimed at harming human populations in different times and different geographical sites.

1.2 Microbes and Humans Interacting: Defense Strategies of Multicellular Live Beings

Life began on Earth *circa* 3.5 billion years ago with unicellular beings: primitive bacteria and algae (*Archean Age*) See: Archea, Wikipedia (accessed 1 Feb 2017) and De Duve [1]. Biological evolution of species progressively produced today's forms of life, as we observe them. Millions of species co-exist today and share the stage (biosphere). Evolution soon registered colonies of unicellular beings, with the advantage of wider and specialized cellular functions but with the problem of preventing undesirable aggregations (casual or purposely parasitic and potentially dangerous situations anyway). Consequently, the need of surveillance by an efficient “immune system” is always present. The basic need of this surveillance system was (and always is) to preserve the genetic integrity of the individual being and the survival of the colony (multicellular entity): any novelty cannot be “tolerated” and regular cells (“self”) must be quickly recognized as such, while stranger cells (“not self”) must be eliminated (rejected) efficiently [2, 3].

In the evolutionary enactment of these needs, anatomical barriers developed, like surface structures (skin, barks, cuticles) and recognition systems (surface molecules, histocompatibility antigens, intercellular communication and collaboration/rejection functions) became very sophisticated, demanding and by necessity error-free. For a general description of the immune system, we recommend reading appropriate textbooks or specialty reference manuals [2, 4]. We can mention in this respect a recent introductory manual by Actor [3]; this book had also an Italian Edition by Pearson Publisher, 2015, “Introduzione all'immunologia”, cared by our scientific team.

In presence of the enormous variety of live beings coexisting nowadays on Earth, humans (we) pretend to have absolute priority, but we have to consider that we share the stage in such a crowded environment on this planet. Therefore, we should adhere to the following principles: learn, respect, understand and prevent. LEARN the biological requirements of all the life forms, RESPECT the right of any form of life for existence and prosperity, UNDERSTAND the logic of evolution and of biological mechanisms and PREVENT the possibilities of harm and disease for humans and other animals and plants of interest, due to environmental changes (mostly man-determined).

On this planet there are millions of different species of living beings, in variable proportions in the biosphere and in different ecosystems: they are all co-existing, interacting and competing for food and survival. This encompasses the very universal phenomena of competition and of parasitism [2].

One of the most recent and precise evaluations of the number of species existing on planet earth, but still very approximate and provisional (well-illustrated and summarized in National Geographic, april 2013), finds evidence of more than five thousand species of mammals, ten thousand species of birds, twelve thousand species of reptiles, fifteen thousand species of amphibians, forty-five thousand species of fish, one hundred and fifty thousand species of crustaceans, two hundred thousand species of mollusks, six hundred thousand species of arcnids, five million species of insects and many, many millions (inestimable indeed) species of bacteria, viruses and other microorganisms. Are we numerous on Earth? Is there enough work for the immune system of each living multicellular organism to distinguish “self” from possibly harmful “not self”?

Species of microorganisms ascertained as pathogenic for humans are indeed a very small fraction of the existing ones: we come to know them better because we study the diseases connected with each of the major pathogens, but we ignore a lot about the greater number of other, presumably innocuous species of microorganisms.

In general, different species interacting may set a parasitic relationship, in which the larger biological entity (*the host*) may receive harm (food loss or disease) and the smaller one (*the parasite*) may get advantages (more food, protection). Both must preserve their identity and prevent contamination by foreign genetic material (immunologic surveillance, bilaterally). Some parasites can even live within the hosts (endoparasitism), like some bacteria and all viruses.

Three types of interactions may occur between a microorganism and a human host: (a) symbiotic relationship, in which the microorganism and the host both benefit; (b) commensal relationship, in which the microorganism gains but the host suffers no harm; and (c) a true parasitic relationship, in which the microorganism gains and the host is harmed (with or without disease) [2].

Symbiosis offers frequently mutual advantages and remains very stable in time. Pathogens (as already mentioned) are a minimal proportion of the existing microorganisms. We humans host some advantageous bacterial populations (in the intestine, on the skin, with commensal germs in the mouth and in genital mucosae): we indeed are also *made of the germs living in/on our body*. *Indeed*, only one cell out of ten in our body is a human cell: the rest are bacteria (the so-called microbiome). Among these, we count billions of bacteria in the intestines, definitely useful for many functions (vitamin production, competition with true pathogens, contribution to metabolism, etc) [4].

The balance between host and parasites depends on two basic forces, an aggressive force by the parasite depending on survival/proliferation/invasion capacity of the parasite itself and a defensive force by the host, depending on the immune mechanisms (phagocytosis, cellular and humoral immune reactions). In this balancing of opposite forces the parasitic relationship is played by the contenders. *If we have marked prevalence of the parasite*, we may have disease (and eventually death) of the host, but *if we have definite prevalence of the host defences*, we may have control (and eventually elimination) of parasites. For a wider general illustration of these concepts see Abbas et al. [2] and also Bologna and Lepidi [5].

1.3 Immunology: Origins and Development of a Still Young Discipline

Immunology is a rather young science (a little more than 60 years old, with this explicit name) although the first informations on the existence and validity of immune defences go back indeed to Edward Jenner with his “vaccination” practices in the 1790s and probably to some older but analogous Chinese medicine practices [6].

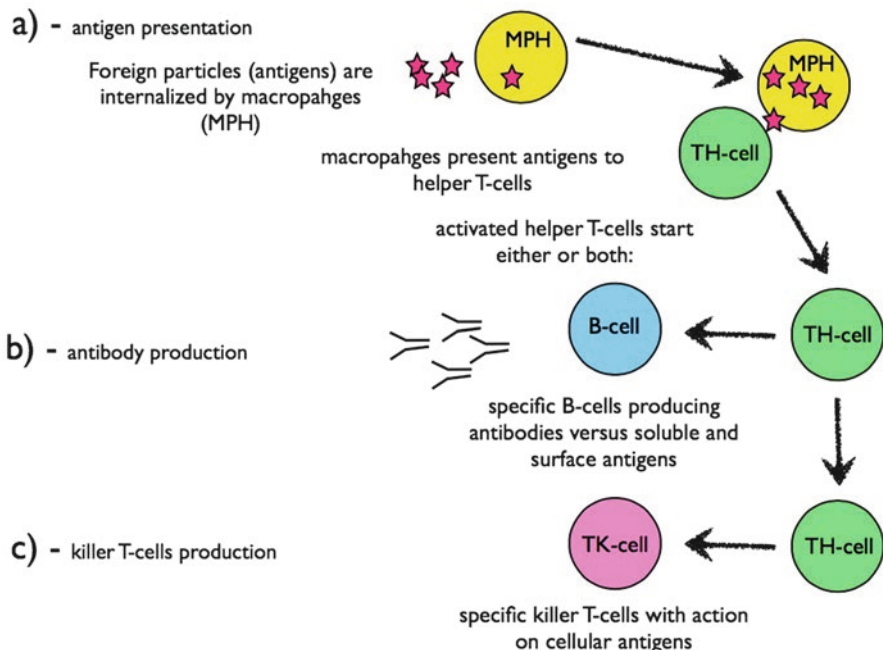
The first “immunological” experiment by Jenner is something that would not be ethically feasible today by any medical deontological rules! He started from the observation that English milkmaids who caught cowpox (a benign form of skin eruptions from contacts with diseased cows) did not develop the human form of the smallpox disease; therefore, he voluntarily and deliberately exposed his gardener's son (James Phipps) to biological materials from cowpox pustules (causing a fever illness in the recipient); after recovery, Jenner challenged the boy with human smallpox, verifying his attained “immunity” also to the human disease [3].

Jenner is therefore considered the father of experimental “immunology”. No mechanistic explanations of that experiment were however possible at that time: only about 100–150 years later we started discovering antibodies and immune system functions allowing us to understand what was biologically happening at that time in the milkmaids (and at any other time in “immune” individuals) and in the “vaccinated” individuals like Jenner's gardener's son.

All the developing “immunological” science, enriched later through microbiology, biochemistry, physiology and pathology studies in the following decades, recorded more and more details in the functioning of the defense mechanisms of mammals and humans, having the most complex and efficient forms of immunological defense against foreign agents entering the body.

The immune system is in fact like an “eye within” the body, controlling that nothing extraneous is biologically active in each individual organism, and therefore, recognizing effectively “self” from “not-self”, so that replication of self-cells are not contaminated by “foreign” biological agents and that any extraneous biological entity will be eliminated by soluble “light” weapons (antibodies) and by “heavy” killers (immune cells and macrophages, interacting and collaborating in the task).

The key steps of an immune reaction are substantially three: (a) internalization of foreign particles by macrophages (MPH) degrading and “presenting” them to T-Helper lymphocytes (TH-cells) which in turn start an elaborated attack, mostly through either (b) synthesis of soluble “light” weapons (antibodies, humoral response by B-cells) or (c) development of cellular “heavy” weapons (killer T-lymphocytes T-K cells, with cellular or cytotoxic response); in many cases we observe the activation of both responses (b + c), depending on the needs. The key steps of an immune reaction are illustrated in the following figure.



Recently, it has been shown that activated T-cells tend to aggregate, like a swarm of bees, exchanging informations, useful to coordinate the immune response (i.e. to elaborate coordinated defence plans): this is the visual demonstration of the complex molecular dialogue taking place within the various families of immune cells collaborating to the full enactment of defence mechanisms.

When invaders are present in body fluids or in the extracellular domains, like most bacteria, they can be attacked by antibodies, through specific surface recognition. However, when the invaders are instead of the intracellular type, like viruses and some bacteria (like TBC), they must instead be attacked by special killer lymphocytes (cytotoxic TK-cells) which destroy all the self-cells harboring the intruders, together with their content. Nevertheless, viruses anyway can also transit the body fluids; consequently also antibodies are produced against them.

In most responses, both humoral (antibodies) and cellular attacks are deployed. 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.

Details of the immune mechanisms are continuously discovered. So this discipline is still far from being completely described and understood. New details are continuously added and better focused by immunology researchers in time [2].

1.4 Microbes and Their Attack Strategies

If germs are successful to penetrate the body, they enter a 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 often undetected by immune reactions and unreachable by antibodies [7].

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

But fortunately, highly pathogenic germs are very few, considering that there are millions of species of microorganisms in nature. Nevertheless, most of these non-pathogenic organism species to human are easily destroyed by the *Homo sapiens* immune system [3].

The very limited number of effective pathogens is characterized by special properties making them resistant to the immune attacks. Very virulent germs tend however to disappear in the ecosystems, because they kill their sensitive hosts (natural selection): the killer usually disappears together with the killed. This indeed is part of the natural selection of life forms able to coexist and collaborate (through non-lethal parasitic relationships). Therefore, life on Earth continues to thrive and evolve [5].

Peace and reciprocal toleration and coexistence are therefore favoured by natural selection: we may say, the biological strength of peace and cooperation (even before and without human knowledge)!!!

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

More strategies are immune evasion (antigenic change - flu viruses) (Bologna, 2010), complement inhibition, resistance to phagocytosis, or even T_H1 elimination (HIV). The competition grounds are therefore many and variegated [2, 3].

1.5 Prevention to Favour Defence and Immunity

To control infections and favor immune responses, therefore, we may generally try:

- (a) To reduce microorganism proliferation (through the use of bacteriostatic drugs) or kill at least partially the germs (disinfection, use of bactericidal drugs);
- (b) To control the vectors diffusing the infection (insects, arthropods, birds, rats, etc.); this action implies vast territory and international cooperation plans;

- (c) To immunize preventively the potential hosts (vaccinations): this action requires public health planning, technology, costs, cooperation plans and time;
- (d) To administer preformed antibodies (serotherapy): this also requires technology, health system costs and availability of sera (biotechnology production facilities).

Actions against germs imply the use of chemotherapeutic agents (antibiotics: bacteriostatic or bactericidal) having a multitude of action mechanisms and being suitable under precise conditions; germs tend to develop resistance to them; fewer and fewer effective antibiotics are available today, while the discovery of new molecules in this category has been scarce, lately.

Hygienic measures are very effective, well known, applicable easily almost everywhere and can be enacted at low cost, whenever possible.

Campaigns against vectors (insecticides, biological competitors - fungi, bats, genetic manipulation of vectors) can also be very effective (if they are done with a good biological and environmental knowledge of the ecological systems in play).

The consensus on the best possible actions are mostly related to effective vaccination campaigns (if specific and effective vaccines are available and if time allows) and immune sera administration (when available and if time is critical for the disease control).

A vaccine is generally preferable, because it induces an active response in the recipient and an advantageous state of immune memory in the treated population, 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 (heterologous proteins –horse immunoglobulins in humans are antigenic-) and does not have a lasting protection (passive immunization).

Vaccinations have resolved many infectious diseases in recent times, like diphtheria, poliomyelitis, pertussis, measles, (that are no longer deadly threats in many areas of the world). Vaccination against smallpox (variola) has actually extinguished the disease from the planet Earth. Unfortunately, this fact anyway presents a very dangerous problem for future potential bioterrorism actions (as we will discuss in a separate chapter in this book).

Moreover, modern life has created some extra occasions for germs and some “new” diseases: 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 Legionnaires disease (an often fatal lung infection by *Legionella*) is very instructive for microbiologists and epidemiologists: every scholar in this field should study the case [8, 9].

Human behaviours (sexual 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 C (HepC) and HIV viruses. Centralized processing of foods has sometimes diffused a contagion of food-borne infections (*E.coli*).

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: for bacteria we may cite *Cryptosporidium parvum*, *Legionella pneumophyla*, *Campylobacter jejuni*, *Helicobacter pylori*, and for the viruses we may cite Rotavirus, Ebola virus, Zika virus, Hantaan virus, HTLV virus, HIV virus, Herpesvirus-6 and -8, virus Guanarito, virus Sabia, nCoV-MERS virus, to name just some of the most relevant and recent ones [10, 11].

Therefore, we have a lot to learn about new infections and a lot to do to control communicable diseases, also because of the changing human ecosystem.

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