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Immunology

IN VITRO IMMUNITY AGAINST THE SARS-COV-2 WHICH CAUSES COVID-19

Adamya Singh Rana

Student, Nirmala Convent School, Mahananda Nagar, Ujjain, Madhya Pradesh, India.

ABSTRACT Today, the current pandemic of the new coronavirus is no less than havoc to the world. The number of infected people around the world is touching the skies. The sufferings of the people are unimaginable. Scientists around the globe are working tirelessly in search of cure to this deadly disease (covid-19). And the most prominent method is developing a vaccine. Though this method treated many diseases, it also shows some side effects (Vaccines, n.d.)6. But my concept is based on the rule of nature and the key point of evolution, natural selection or survival of the fittest. And the concept says that putting the new coronavirus with the body's immune cells will force them to survive, if not, then it will give them the time to understand the virus and create a permanent memory for the virus. Once the cells get fully immunized against the virus they are ready to be injected into the body for a permanent and effective protection.

KEYWORDS : Corona Virus; covid-19 vaccine; Immunization; In vitro immunity

INTRODUCTION

The immune system defends our body against invaders such as viruses, bacteria etc. Its role is necessary for our survival; it is the strongest and most effective mechanism present in our body which defends us as we drift through a sea of pathogens. At this time when the outbreak of SARS-CoV-2 started as an epidemic and soon become a pandemic, the immune system work is increased. As this is the sudden outbreak of new virus, our immune system is unable to develop an effective immunity against this virus as a result thousands of people died and millions are suffering. So as a permanent way to develop immunity against this virus I put forward my concept. My concept's inspiration is the rule of nature and evolution, that is, if an entity has to survive it has to adapt to its environment. So before we go to goal or main point of this concept we must discuss how the immune system works.

When a pathogen enters our body's immune system begins to battle with the pathogen, once the pathogen is defeated, some T-cells and B-cells turn into Memory T-cells and Memory B-cells, and these cells remember the pathogen they just fought, the memory cells live in the body for a long time, even after all the pathogens from the first infection have been destroyed they stay in the ready-mode to quickly recognize and attack any returning viruses or bacteria. Quickly making lots of antibodies can stop an infection in its tracks. The first time our body fights a pathogen, it can take up to 15 days to make enough antibodies to get rid of it. With the help of Memory B-cells, the second time our body sees that virus, it can do the same in thing 5 days it also makes 100 times more antibodies than it did the first time, the faster your body makes antibodies, the quicker the virus can be destroyed, with the help of Memory B-cells, we might get rid of it before we even feel sick, this is called gaining immunity (Ask a Biologist, n.d.)[1]. In depth functioning of immune system is given below:-

According to a study by Vivian Turner (n.d.)[5], B cells have two main types of immune responses. In a T-Independent immune response B cells can respond directly to the antigen. In a T-dependent immune response the B cells need assistance from T cells in order to respond.

In this situation activated B cells move to the border of the T cell zone to interact with T cells (Figure 1). CD40 ligand is found on these T helper cells and interacts with CD40 on the B cells to form a stable attraction. Cytokines secreted by T cells encourage proliferation and isotype switching and maintain germinal center size and longevity. Without these signals the germinal center response will quickly collapse.

B cells that have encountered antigen and begun proliferating may exit the follicle and differentiate into short-lived plasma cells called plasma blasts (Figure 1). They secrete antibody as an early attempt to neutralize the foreign antigen. They do not survive more than three days but the antibody produced can provide important assistance to stop fast-dividing pathogens such as viruses.

The germinal center has a light zone and a dark zone. The germinal center response begins in the dark zone where the B cells rapidly proliferate and undergo somatic hyper mutation. During somatic hyper mutation, random mutations are generated in the variable domains of the BCR by the enzyme activation-induced cytidine deaminase (AID). B cells then enter the light zone and compete with each other for antigen. If the mutation resulted in a BCR with an improved affinity to the antigen the B cell clone can out-compete other clones and survive. The light zone is also thought to be where B cells undergo class switch recombination, although a germinal center is not crucial for this process. The B cells may migrate between both zones to undergo several rounds of somatic hyper mutation and class switch recombination. The ultimate goal of the germinal center is to produce B cells with a BCR which has high affinity for the initial antigen.

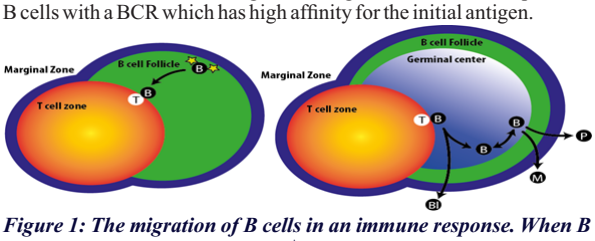

Figure 1: The migration of B cells in an immune response. When B cells (B) first encounter antigen (★) they migrate to the T-B border to receive survival signals from T cells (T). If they receive survival signals they will begin to proliferate and either become plasma blasts (Bl) or form a germinal center (Blue). B cells can migrate between the light zone and dark zone of the germinal center to undergo somatic hyper mutation and class switch recombination. Eventually they may leave the GC as high-affinity memory cells (M) or plasma cells (P).

Plasma and memory cells:-

B cells leave the germinal center response as high-affinity plasma cells and memory B cells (Figure 2). Plasma cells secrete antigen-binding antibodies for weeks after activation. They migrate to the bone marrow soon after formation where they can reside indefinitely, ready to encounter the antigen again and respond. Memory B cells circulate throughout the body on the lookout for antigen with a high-affinity for their BCR and then quickly respond to the antigen, stopping infection. This is how vaccination works. As your body has been previously exposed to the antigen the immune cells can quickly respond to remove the antigen if it is encountered again, stopping you getting sick (Vivian Turner, n.d.)[5].

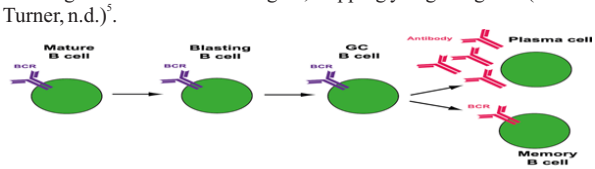

Figure 2: B cell differentiation after activation. When a mature B cell encounters antigen that binds to its B cell receptor it becomes activated. It then proliferates and becomes a blasting B cell. These B cells form germinal centers. The germinal center B cells undergo somatic hyper mutation and class switch recombination. Plasma cells and memory B cells with a high-affinity for the original antigen stimuli are produced. These cells are long lived and plasma cells may secrete antibody for weeks after the initial infection.

Similarly, T-cell activation also takes place when T cells migrate to secondary lymphoid tissues where they interact with antigen, antigen-presenting cells, and other lymphocytes.

When T-cells are activated, cell cycle entry and cell division, clonal expansion, secretion of cytokines (helper cells), activation of killer functions (cytotoxic cells), acquisition of effector function, memory and death occurs which is important for down-regulation of immune response.

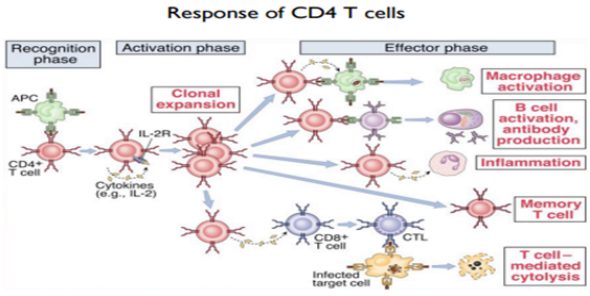

Figure 3: Activation of T-cells.

So by combining this property of formation of memory cells by immune system with the basic rule of nature, we could probably achieve immunity to covid-19. To give my concept a stronger foundation I have taken this reference, a scientist kept some bacteria with PET plastic without giving bacteria its food. After some days the bacteria was eating the plastic as a source of carbon and then using the carbon to make sugar (León-Zayas, 20 June 2019)[4]. This means that the bacteria has adapted to its surrounding.

In a more detailed manner, my plan is to develop immunity to covid-19 in some cells of immune system (PBMCs), in vitro, by keeping them with SARS-CoV-2 over some time. In this period the cells get enough time to understand, develop immunity and overrule the virus, as these cells gets "adapted" to their surroundings. Once the cells get immune they are ready to be injected into a person's body for permanent protection against covid-19, thus ending the pandemic.

MATERIALS AND METHODS

Materials required:

Density gradient medium, phosphate buffered saline, L-leucyl-L-leucine methyl ester, lipopolysaccharide, SARS-CoV-2 virus or its spike protein.

METHODS:

- **Isolation**

PBMCs (Peripheral Blood Mononuclear Cells) are isolated by density gradient centrifugation, as different components of the blood have different densities and can be separated accordingly. The density gradient medium most commonly used (Ficoll or Ficoll-Paque) contains sodium diatrizoate, polysaccharides, and water, and has a density of 1.08 g/mL. This medium is denser than lymphocytes, monocytes, and platelets (meaning these will remain above it), but less dense than granulocytes and erythrocytes, which will drop below it (Barnabe, 30 May 2017)[2].

To isolate PBMCs, whole blood, diluted with phosphate-buffered saline (PBS), is gently layered over an equal volume of Ficoll in a Falcon tube and centrifuged for 30-40 minutes at 400-500 g without brake. Four layers will form, each containing different cell types—the uppermost layer will contain plasma, which can be removed by pipetting. The second layer will contain PBMCs and is a characteristically white and cloudy "blanket." These cells can be gently removed using a Pasteur pipette and added to warm medium or PBS to wash off any remaining platelets. The pelleted cells can then be counted and the percentage viability estimated using Trypan blue staining (Barnabe, 30 May 2017)[2].

The PBMCs which directly isolated from human blood cannot show the sensitization to antigens because of the suppressed function from the immunosuppressive cells such as monocytes and NK cells. However, after the treatment with L-leucyl-L-leucine methyl ester (LLME), the PBMCs are able to initiate the immune response against any antigens as the immunosuppressive cells have been removed (Creative Biolabs, n.d.)[3]. After treating it with the LLME, dendritic cell must be added to it as to present antigen to the B and T cells.

- **Culturing**

PBMCs can be cultured for 5-7 days in 24- or 96-well plates, using supplemented RPMI-1640 medium, and incubated at 37°C in a humidified, 5% CO2 atmosphere. PBMCs do not readily proliferate without stimulus and should be plated at a density of 0.5-1 x 106 cells/mL in a total volume of 0.5-1 mL in a 24-well plate, and 200 µL in a 96-well plate. Because PBMCs comprise both lymphocytes and monocytes, some cells will adhere to the plate (monocytes/macrophages) while others may be in suspension (lymphocytes). This must be taken into account when culturing—for example, round- or V-bottom plates may be more appropriate if cells need to be pelleted by centrifugation. To induce proliferation, phytohemagglutinin (PHA) or lipopolysaccharide (LPS) can be added to the culture medium at a concentration of 1-5 µg/mL (Barnabe, 30 May 2017)[2], with the different strains of SARS-CoV-2.

- After doing this we just have to wait and give proper conditions and nutrition to the PBMCs.

RESULTS AND DISCUSSION

The probable result after doing these things could be that the lymphocytes mainly T and B cells interacted with the virus and developed into memory cells, and as this happens, they are ready to be injected into the body for permanent immunity.

CONCLUSION

As a conclusion to this hypothesis, the use of in vitro immunity against a microorganism is better than the use of vaccines. As it is safer, effective and probably permanent. Moreover, further modifications and experiments could be done like genetically modifying the cells or cell reprogramming, etc.

REFERENCES

1. Ask A Biologist. (n.d.). Askabiologist.Asu.Edu. Retrieved August 4, 2020, from https://askabiologist.asu.edu/memory-b-cell
2. Barnabe, M. (2017, May 30). Peripheral blood mononuclear cells: PBMC isolation, preservation, and culture. Quartzy. https://blog.quartzy.com/2017/05/30/peripheral-blood-mononuclear-cells-pbmc-isolation-preservation-culture
3. Creative Biolabs. (n.d.). Antibody production by in vitro immunization. Retrieved September 10, 2020, from https://www.creative-biolabs.com/antibody-production-by-in-vitro-immunization. html#: ~:text =Creative % 20 Biolabs % 20 can % 20 make % 20 use,antigens%20during%20the%20vaccines%20test
4. León-Zayas, R. (2019, June 20). Draft Genome Sequences of Five Environmental Bacterial Isolates That Degrade Polyethylene Terephthalate Plastic. Microbiology Resource Announcements. https://mra.asm.org/content/8/25/e00237-19.full
5. Turner, V. T. (n.d.). B cell activation and the germinal centre response. British Society for Immunology. Retrieved September 10, 2020, from https:// www. immunology. org/public-information/bitesized-immunology/immune-development/ b-cell-activation-and-the-germinal-centre
6. Vaccines. (n.d.). Vaccine Side Effects. Vaccines.Gov. Retrieved August 4, 2020, from https://www.vaccines.gov/basics/safety/side_effects