Team member

Instructor: Yasuhiro Iida

Ayumi kida, Azusa Nomura, Takuya Ohyama, Shinichirou Ida, Rihou Sasaki, Toshitaka Nishimura, Miyuki Saijo, Sachina Tosu, Nozomi Suzuki, Shota Maeda, Kunifumi Kanai and more...

Hay fever curE.coli

Back ground

Japan, one of six person is troubled now by hay fever. These people take a medicine for the hay fever. But, when they take the medicine, several side effect of the medicine will appears, for example become sleepy, thirsty, lazy and so on. If become sleepy, they cannot work and study. So, we were working on a project to relieve hay fever by Escherichia coli to improve these problem. It is two kinds of immune cells said to be Th1 cell and Th2 cell to greatly participate in hay fever, and both usually keep balance and control immunoreaction. When biased to Th2 cell immune balance between Th2 cells and Th1 cells is lost due to changes in the living environment and diet, IgE antibodies that react with allergens such as pollen is produced in excess, allergy symptoms are from being developed . Further, those two kinds of the cells have also been found that in order to balance the immune whole mutually restrain each other. Therefore, to increase the amount of Th1 cells than Th2 cells, it is possible to restrain the Th2 cells, and suppress the production of IgE antibodies against substances less harm that may be unresponsive nature such as pollen and allergies it is thought to be improved.

Immune system

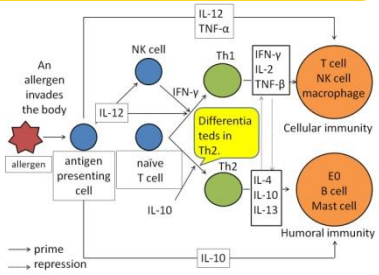
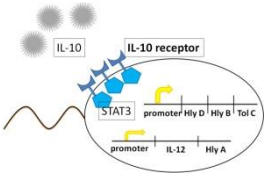


Figure1 Immune system

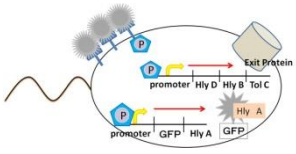
Immune responses are regulated firstly by antigen-presenting cells,such as the macrophages and dendritic cells ,and by the T helper lymphocyte subclasses Th1 and Th2, which are components of acquired immunity. Naive Th0 cells are clearly bipotential and serve as precursors of Th1 and Th2 cells. Among the factors currently have been known to influence the differentiation of these cells towards the Th1 or Th2 subset, the cytokines produced by cells of the innate immune system are the most important. Thus, IL-12, produced by activated macrophages or other Allergens, is the major inducer of Th1 differentiation and hence cellular immunity. This cytokine acts in concert with natural killer (NK)-cell-derived IFN-γ to promote Th1 responses. IL-12 and TNF-α, in concert with NK- and Th1-cell-derived IFN-γ stimulate the functional activity of T cytotoxic (Tc) cells, NK cells and activated macrophages, which constitute the major components of cellular immunity. Because of these crucial and synergistic roles in inflammation, IL-12, TNF-α and IFN-γ are considered the major pro-inflammatory cytokines. Th1 and Th2 responses are mutually inhibitory. Thus, IL-12 and IFN-γ inhibit Th2, and IL-4 and IL-10 inhibit Th1 responses. IL-4 and IL-10 promote humoral immunity by stimulating the growth and activation of mast cells and eosinophils, the differentiation of B cells into antibody-secreting B cells and B-cell immunoglobulin switching to IgE. Importantly, these cytokines inhibit macrophage activation, T-cell proliferation and the production of pro-inflammatory cytokines. Thus, IL-4 and IL-10 are the major anti-inflammatory cytokines. Th1 and Th2 responses are mutually inhibited. Thus, IL-12 and IFN-γ inhibit Th2, and IL-4 and IL-10 inhibit Th1 responses. IL-4 and IL-10 promote humoral immunity by stimulating the growth and activation of mast cells and eosinophils, the differentiation of B cells into antibody-secreting B cells and B-cell immunoglobulin switching to IgE. Importantly, these cytokines inhibit macrophage activation, T-cell proliferation and the production of pro-inflammatory cytokines. Thus, IL-4 and IL-10 are the major anti-inflammatory cytokines.

Therefore, when receiving the IL10 using the immune system, we will make a device for producing IL12. And above Th2 cells in the body, increase the cells of Th1 cells. We thought it suppress the reaction of hay fever.

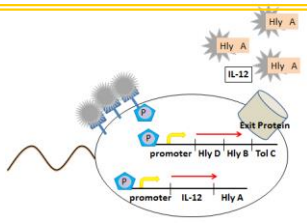
We are making E.coli which has ability to cure hay fever.
We will give following functions to E.coli.



1. Expression of IL-10 receptor to E.coli.



2. Phosphorylation of STAT3.
3. Preparation of gene array with HlyA and L-12 promoter and receiving the STAT3.

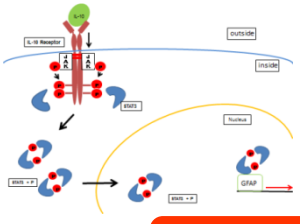


4. Preparation of gene array with TolC and HlyB and HlyD promoter and to receive the STAT3.

The details of our project
We wanted to try to create a device, as shown in Figure 2.

(1) Interleukin10 receptor
IL10 receptor receives IL10. IL10 receptor consists of two kinds, α chain and β chain. And it has taken the structure of the dimer. IL10 is increased in the body, IL10receptor will be receiving first.

(2)STAT3(Signal Tranducer and Activator of Transcription 3)
Stat3 is a signal and transcription factors in the cell.It is present in the cytoplasm in the deactivation mode IL10 binds to the IL10 receptor, intracellular domain of JAK（Janus Kinas）receptor is phosphorylated STAT3. It shifts to the nucleus to form a dimer that STAT3 is phosphorylated. STAT3 dimers are transferred into the nucleus, and activates the transcription by binding to the GFAP gene as a target.



(3) Downstream gene
Phosphorylated STAT3 binds to the GFAP gene promoter. GFAP has a phosphorylated STAT3 binding site. Channel for the transport protein and gene of interest is created when it binding. It is a mechanism that IL12 is released to outside bacteria. This system was created based on the draft of 2011 the UNICAMP-EMSE.
IL12 activity test is difficult in the short term, it was thought that instead of the GFP. It would be replaced by IL12 if successful.

Human practice

1.Overview

We did questionary survey about the gene recombination to our high school students. As a result of questionnaire, we could know that there were many people who had a bad image for gene recombination. “How do you think of gene recombination?” Answer result It is
・adverse effects to a body.
・It is untrustworthy.
・It is difficult.
We thought that we wanted many people to know the good point of genetic modification and the synthetic creature.

2.Posters

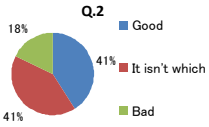
We want to convey that experiment of a gene recombination is not a dangerous thing. We made the poster of the question and answer form to make general people understand it. At first we felt that understanding about the reason why experiment of a gene recombination is dangerous for general people didn't have enough information. Therefore we commented on the risk of the gene recombination experiment from a point of view of the diffusion the recombination creature to the environment. And we gave the following examples and explained that there was an international decision for nonproliferation of the gene recombination creature to the environment.
There are P1, P2, P3 level in the laboratory where we experiment of gene recombination.
There is Cartagena Protocol about the handling of the gene recombination creature.
Our university has the laboratory of P1 and P2 level. We explained to a general person that we abide for nonproliferation of the genetically-modified creature to the environment. And we made the poster about the activity contents of the iGEM.

3.Questionnaire

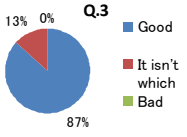
Our survey consisted of the following

Q1.Are you interested in genetic modification?

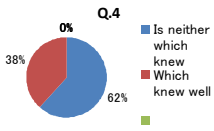
Q2.What kind of impression did you have about genetic modification before hearing this explanation?



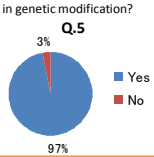
Q3.After having heard this explanation, what kind of impression did you have about genetic modification?

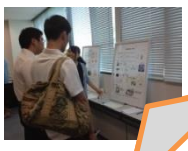


Q4.Was our explanation plain?



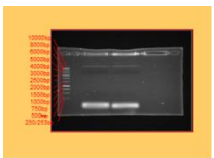
Q5.Were you interested in genetic modification?





Results

We inserted IL-10 recepter gene, GFP gene , stat3 and HlyA in vecter.
We experimented as following.



1. Transformation.
2. Culture on the LB nutrient medium and get colonies.
3. Colony PCR.
4. Restriction enzyme processing PCR production.
5. Infusion.
6. Transformation.
7. Culture on the LB nutrient medium and get colonies.
8. Colony PCR.

We were trying to make the parts. However, it was impossible to complete.
1. IL10 receptor device (BBa_K1069002)
2. STAT3 (Signaling and transcriptional activator) (BBa_K1069003)
3. GFP＋HlyA device (BBa_K1069004)
4. GFAP + GFP + HlyA device (BBa_K1069005)