



Adherence
Part II – Identification of Host Cell Receptors
Teacher instructions

Teacher Prep

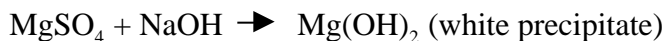
In order to make this experiment simpler, I designed a precipitation reaction to mimic the agglutination reaction.

Preparation

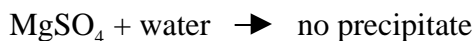
Make 500 mls of each solution below

Solution A – 1 M MgSO_4

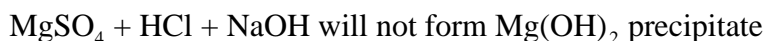
Solution B – 0.5 M NaOH



Solution C – water



Solution D – 1 M HCl



(A brief precipitate may form upon addition of NaOH before mixing with the toothpick)

Distribute solutions A-D into bottles as follows. Very little solution is needed in each bottle. Label each bottle with the scenario number. **One** of each dropper bottle is needed.

Scenario 1

Bladder cells (solution A) MgSO₄
E.coli with type I fimbrae (Solution B) NaOH
Glucose (Solution C) water
Mannose (Solution D) HCl
Galactose (Solution C)
Fructose (Solution C)

Scenario II

Bronchial Cells NCI-H292 (Solution A) MgSO₄
Wild Type Bordetella (Solution B) NaOH
Fimbriae mutant (Solution B)
Filamentous Hemagglutinin mutant (Solution C) water
Pertussis Toxin mutant (Solution B)
Hemolysin mutant (Solution B)
Adenylate Cyclase mutant (Solution B)

Scenario III

Laryngeal Cells HEP-2 (Solution A) MgSO₄
Wild Type Bordetella (Solution B) NaOH
Fimbriae mutant (Solution C) water
Filamentous Hemagglutinin mutant (Solution C)
Pertussis Toxin mutant (Solution B)
Hemolysin mutant (Solution B)
Adenylate Cyclase mutant (Solution B)

Scenario IV

T-cells (Solution A) MgSO₄
HIV (Solution B) NaOH
Anti-CD3 (Solution C) water
Anti-CD4 antibody (Solution D) HCl
Anti-CR5 (Solution C)
Anti CRCX4 (Solution D)

Possible Answers and Experimental Designs

Scenario I

Possible Hypotheses

Mannose is the receptor on bladder cells for *E. coli* with type I pili
Galactose is the receptor on bladder cells for *E. coli* with type I pili
Etc

Prediction for first hypothesis above

If mannose is the receptor, it will inhibit agglutination.

Experimental design

Bladder cells + mannose + *E. coli*
Bladder cells + galactose + *E. coli*
Bladder cells + glucose + *E. coli*
Bladder cells + fructose + *E. coli*

Negative controls
Bladder cells alone
Each sugar alone
E. coli alone

Positive control
Bladder cells + *E. coli*

Note – if you add the *E. coli* to the bladder cells before adding the sugar, the sugar will not be able to inhibit the reaction and agglutination will be observed.

Answer –

Mannose is the receptor for this type of fimbriae and should inhibit agglutination in this test

Scenarios II and III

Note

The filamentous hemagglutination mutant is a strain lacking filamentous hemagglutinin etc
The *B. pertussis* wild type strain is a strain with all possible adhesins present

Possible Hypotheses

Filamentous hemagglutinin is responsible for the adherence of *B. pertussis* to bronchial/laryngeal cells.

Fimbriae are responsible for the adherence of *B. pertussis* to bronchial/laryngeal cells

Etc

Prediction based on the first hypothesis

If filamentous hemagglutinin is responsible for the adherence of *B. pertussis* to bronchial/laryngeal cells then a mutant lacking filamentous hemagglutinin will not cause agglutination.

Experimental

Filamentous hemagglutinin mutant + bronchial/laryngeal cells

Fimbriae mutant + bronchial/laryngeal cells

Pertussis Toxin mutant + bronchial/laryngeal cells

Hemolysin mutant + bronchial/laryngeal cells

Adenylate Cyclase mutant + bronchial/laryngeal cells

Negative controls

Each bacterial strain alone

Bronchial or laryngeal cells alone

Positive control

B. pertussis wild type + bronchial/laryngeal cells

Answer

For scenario II – binding to bronchial cells is mediated by filamentous hemagglutinin (no agglutination observed with the FHA mutant)

For scenario III – binding to laryngeal cells is mediated by fimbriae and filamentous hemagglutinin (no agglutination observed with the FHA mutant or the fimbriae mutant)

Scenario IV

Possible hypotheses

CD3 is the co-receptor for HIV on T cells

CXCR4 is the co-receptor for HIV on T cells

Prediction for first hypothesis

If CD3 is the co-receptor for HIV on T cells then antibodies to CD3 should inhibit agglutination.

Experimental

T-cells + anti-CD3 + HIV

T-cells + anti-CRCX4 + HIV

Positive controls

T-cells + HIV

Negative controls

T-cells alone

HIV alone

Other controls

T-cells + anti-CD4 + HIV (expect no agglutination confirming that CD4 is part of co-receptor – ie both CD4 and another molecule serve together as the receptor)

T-cells + anti-CCR5 + HIV (expect no agglutination confirming that CCR5 is not involved in binding – this is expected since it is thought that there is no CCR5 on T-cells)

Note – if you add the HIV to the T-cells before adding the antibody, the antibody will not be able to inhibit the reaction and agglutination will be observed.

Answer

CRCX4 is the co-receptor for HIV on T-cells (antibodies to CRCX4 inhibited agglutination).