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

$MgSO_4$ + NaOH → $Mg(OH)_2$ (white precipitate)

Solution C – water

$MgSO_4$ + water → no precipitate

Solution D – 1 M HCl

$MgSO_4$ + HCl + NaOH will not form $Mg(OH)_2$ precipitate

(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) $MgSO_4$
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) $MgSO_4$
Wild Type Bordetella (Solution B) NaOH
Fimbrae mutant (Solution B)
Filamentous Hemagglutanin 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) $MgSO_4$
Wild Type Bordetella (Solution B) NaOH
Fimbrae mutant (Solution C) water
Filamentous Hemagglutanin mutant (Solution C)
Pertussis Toxin mutant (Solution B)
Hemolysin mutant (Solution B)
Adenylate Cyclase mutant (Solution B)

Scenario IV
T-cells (Solution A) $MgSO_4$
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*
Baldder 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 fimbrae and should inhibit agglutination in this test

Scenarios II and III

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

Possible Hypotheses
Filamentous hemagglutanin is responsible for the adherence of *B. pertussis* to bronchial/larneal cells.
Fimbrae are responsible for the adherence of *B. pertussis* to bronchial/larngeal cells
Etc

Prediction based on the first hypothesis
If filamentous hemagglutanin is responsible for the adherence of *B. pertussis* to bronchial/larngyl cells then a mutant lacking filamentous hemagglutanin will not cause agglutination.

Experimental

Filamentous hemagglutanin mutant + bronchial/laryngeal cells
Fimbrae 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 hemagglutanin (no agglutanation observed with the FHA mutant)
For scenario III – binding to laryngeal crlls is mediated by fimbrae and filamentous hemagglutanin (no agglutanation observed with the FHA mutant or the fimbrae 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 contorls
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).