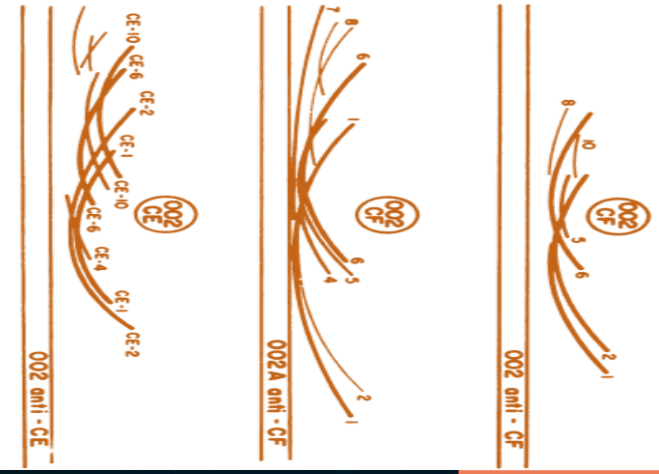
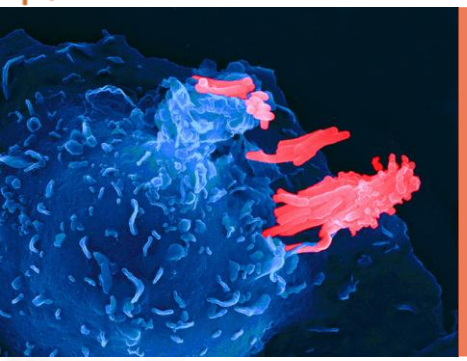


# Yeni mikobakteriyel antijenler ve bunlara karşı konak immün yanıt

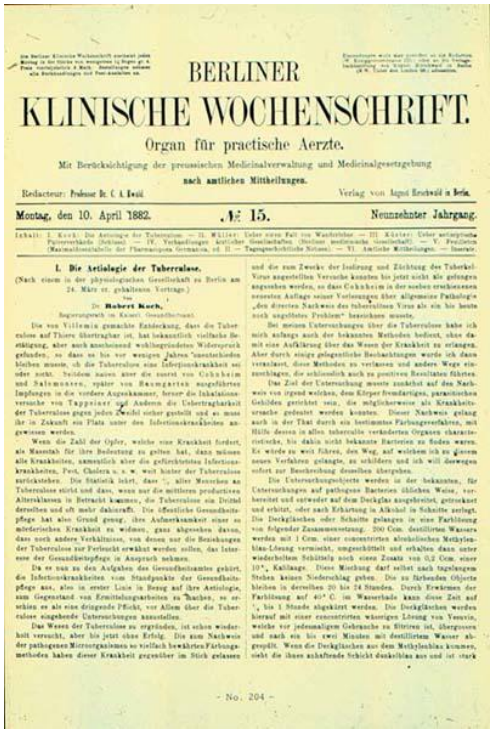

Barış Otlu
İnönü Üniversitesi Tıp Fakültesi
Tıbbi Mikrobiyoloji Anabilim Dalı


IL-12

# İlk aşı araştırmaları

- Tüberküloz ile ilgili ilk aşı çalışmaları,

  etkenin 1882'yılında Koch tarafından tanıtılmasından hemen sonra başladı.



BERLINER

KLINISCHE WOCHENSCHRIFT.

Organ für practische Aerzte.

Mit Berücksichtigung der preussischen Medicinalverwaltung und Medicinalgesetzgebung nach amtlichen Mittheilungen.

Redacteur: Professor Dr. C. A. Ewald — Verlag von August Hirschwald in Berlin.

Montag, den 10. April 1882. № 15. Neunzehnter Jahrgang.

I. Die Aetiologie der Tuberculose.

(Nach einem in der physiologischen Gesellschaft zu Berlin am 24. März cr. gehaltenen Vortrage.)

Dr. Robert Koch,

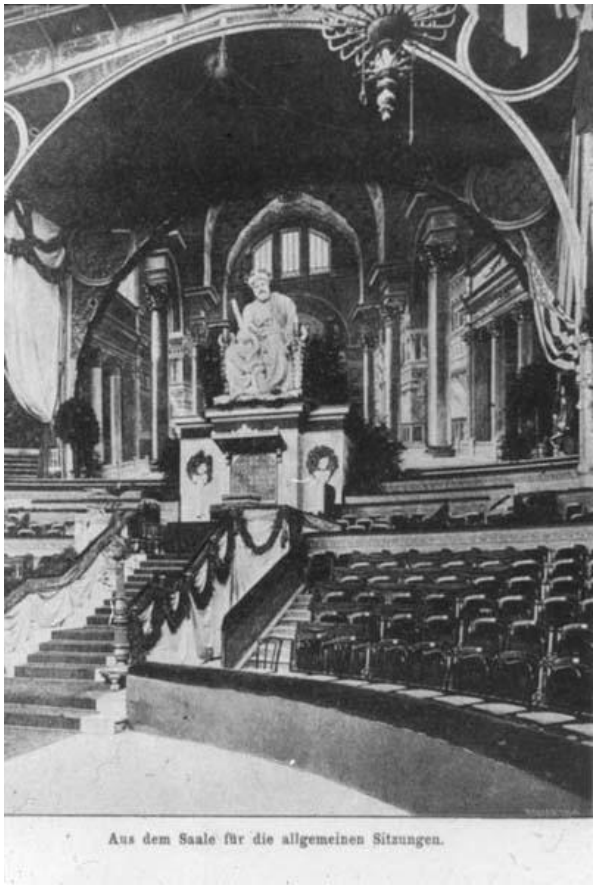
Regierungsrath im Kaiserl. Gesundheitsamt.

A published version of Koch's lecture "The etiology of tuberculosis" in *Berliner Klinische Wochenschrift* 10 April 1882.

Robert Koch

# İlk Aşı Araştırmaları

- Koch 1890 yılında mikobakterilerden «tuberculin antijenini» ekstrakte ettiğini ve içeriğini açıklamadığı bu kompozisyonla tüberkülozu tedavi ettiğini duyurmuştur.




R. Koch.

# İlk Aşı Araştırmaları

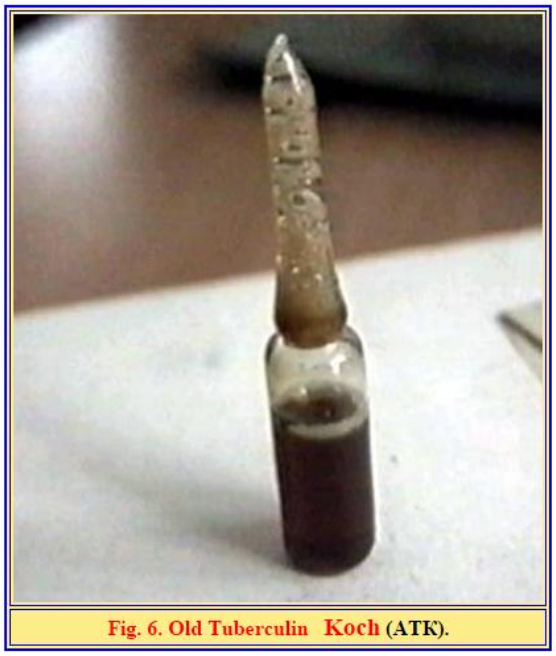


Fig. 6. Old Tuberculin **Koch** (ATK).


A contemporary sketch demonstrating the treatment of a tuberculosis patient with tuberculin.

# İlk Aşı Araştırmaları

Die Wirksamkeit

des

Koch'schen Heilmittels gegen Tuberkulose.

Amtliche Berichte

der

Kliniken, Polikliniken und pathologisch-anatomischen Institute

der Preussischen Universitäten.

Ausgesondert

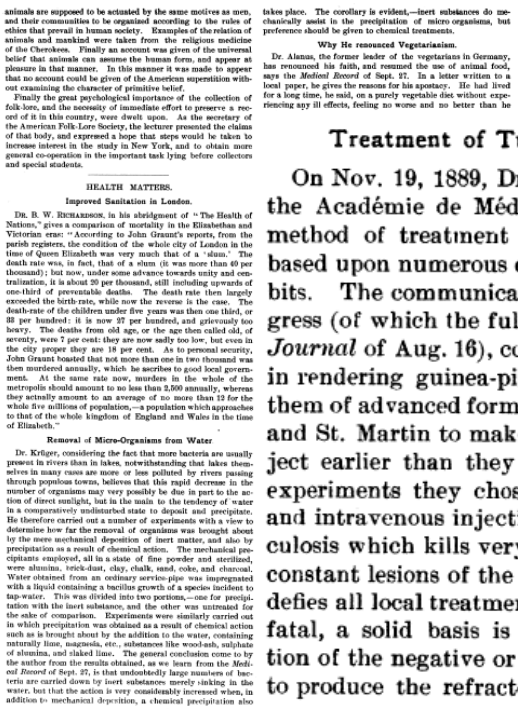
Mit einer Zusammenstellung der Berichtsergebnisse

von Professor Dr. Albert Gottstadt.

Berlin.

Verlag von Julius Springer.

1891.



OCTOBER 3, 1890.] SCIENCE. 191

animals are supposed to be actuated by the same motives as men, and their communities to be organized according to the rules of ethics that prevail in human society. Examples of the relation of animals and mankind were taken from the religious medicine of the Cherokees. Finally an account was given of the universal belief that animals can assume the human form, and appear at pleasure in that manner. In this manner it was made to appear that no account could be given of the American superstition without examining the character of primitive belief.

Finally the great psychological importance of the collection of folk-lore, and the necessity of immediate effort to preserve a record of it in this country, were dwelt upon. As the secretary of the American Folk-Lore Society, the lecturer presented the claims of that body, and expressed a hope that steps would be taken to increase interest in the study in New York, and to obtain more general co-operation in the important task lying before collectors and special students.

HEALTH MATTERS.

**Improved Sanitation in London.**

DR. B. W. RICHARDSON, in his abridgment of "The Health of Nations," gives a comparison of mortality in the Elizabethan and Victorian eras: "According to John Graunt's reports, from the parish registers, the condition of the whole city of London in the time of Queen Elizabeth was very much that of a 'slum.' The death rate was, in fact, that of a slum (it was more than 40 per thousand); but now, under some advance towards unity and centralization, it is about 20 per thousand, still including upwards of one-third of preventable deaths. The death rate then largely exceeded the birth-rate, while now the reverse is the case. The death-rate of the children under five years was then one third, or 33 per hundred: it is now 27 per hundred, and grievously too heavy. The deaths from old age, or the age then called old, of seventy, were 7 per cent: they are now sadly too low, but even in the city proper they are 18 per cent. As to personal security, John Graunt boasted that not more than one in two thousand was then murdered annually, which he ascribes to good local government. At the same rate now, murders in the whole of the metropolis should amount to no less than 2,500 annually, whereas they actually amount to an average of no more than 12 for the whole five millions of population,—a population which approaches to that of the whole kingdom of England and Wales in the time of Elizabeth."

**Removal of Micro-Organisms from Water**

Dr. Krüger, considering the fact that more bacteria are usually present in rivers than in lakes, notwithstanding that lakes themselves in many cases are more or less polluted by rivers passing through populous towns, believes that this rapid decrease in the number of organisms may very possibly be due in part to the action of direct sunlight, but in the main to the tendency of water in a comparatively undisturbed state to deposit and precipitate. He therefore carried out a number of experiments with a view to determine how far the removal of organisms was brought about by the mere mechanical deposition of inert matter, and also by precipitation as a result of chemical action. The mechanical precipitants employed, all in a state of fine powder and sterilized, were alumina, brick-dust, clay, chalk, sand, coke, and charcoal. Water obtained from an ordinary service-pipe was impregnated with a liquid containing a bacillus growth of a species incident to tap-water. This was divided into two portions,—one for precipitation with the inert substance, and the other was untreated for the sake of comparison. Experiments were similarly carried out in which precipitation was obtained as a result of chemical action such as is brought about by the addition to the water, containing naturally lime, magnesia, etc., substances like wood-ash, sulphate of alumina, and slaked lime. The general conclusion come to by the author from the results obtained, as we learn from the *Medical Record* of Sept. 27, is that undoubtedly large numbers of bacteria are carried down by inert substances merely sinking in the water, but that the action is very considerably increased when, in addition to mechanical deposition, a chemical precipitation also takes place. The corollary is evident,—inert substances do mechanically assist in the precipitation of micro organisms, but preference should be given to chemical treatments.

**Why He renounced Vegetarianism.**

Dr. Alanus, the former leader of the vegetarians in Germany, has renounced his faith, and resumed the use of animal food, says the *Medical Record* of Sept. 27. In a letter written to a local paper, he gives the reasons for his apostacy. He had lived for a long time, he said, on a purely vegetable diet without experiencing any ill effects, feeling no worse and no better than he

## Treatment of Tuberculosis by the Vaccine Method.

On Nov. 19, 1889, Drs. J. Grancher and St. Martin addressed to the Académie de Médecine, Paris, a sealed packet relating to a method of treatment and preventive inoculation of tuberculosis based upon numerous experiments which they had made on rabbits. The communication made by Dr. Koch to the Berlin Congress (of which the full text was published in the *British Medical Journal* of Aug. 16), concerning the results which he has obtained in rendering guinea-pigs refractory to tuberculosis, or in curing them of advanced forms of tuberculosis, has induced MM. Grancher and St. Martin to make known their researches on the same subject earlier than they would otherwise have done. In all these experiments they chose the rabbit as the subject of inoculation and intravenous injection, because there is thus produced a tuberculosis which kills very quickly, and at an almost fixed date, with constant lesions of the liver, the spleen, and the lungs, and which defies all local treatment. Tuberculosis thus induced being always fatal, a solid basis is thus secured which allows exact appreciation of the negative or positive results of any method which tends to produce the refractory state or to cure after infection. The

# İlk Aşı Araştırmaları

- Tuberculin tedavi etmiyor.

Rudolf Virchow



which increased the uniformity of the preparation (Pottenger 1913) and avoided the variations in concentration of the active principle (Sahli 1912).

The physicians recommended the treatment and patients themselves bought the remedy to be administered, and even if it was cheap compared to other treatment, it still was expensive for the poor, which were the population collective more susceptible to need it (Salvat-Papasseit 2007).

"Tuberculin is quite harmless, if there is no tuberculosis". Wilkinson

"Tuberculin is not a poison". Pottenger

"The best rule to follow: better too little than too much tuberculin!". Sahli

"The physician of the future will, I foresee, take upon himself the rôle of an immunizator". Wright

Fig. 3. Quotes on tuberculin treatment, published in the manuscripts of that time.

# İlk Aşı Araştırmaları

- “Eski Tüberkülin” ve “Yeni Tüberkülin”

## A Lecture
ON
## THE TREATMENT OF TUBERCULOSIS BY DIFFERENT KINDS OF TUBERCULIN.

*Delivered at the Medical Graduates' College and Polyclinic, London, on Nov. 12th, 1907,*

BY NATHAN RAW, M.D., M.R.C.P. LOND., F.R.S. EDIN.,

PHYSICIAN TO THE MILL ROAD INFIRMARY, LIVERPOOL; MEMBER OF THE INTERNATIONAL COMMITTEE FOR THE PREVENTION OF CONSUMPTION, ETC.

GENTLEMEN,—The treatment of phthisis pulmonalis and other forms of tuberculosis is of such intense urgency and importance that any method based on scientific investigation ought to be thoroughly tested with a view to diminish the awful mortality in this country from tuberculosis. I have been greatly disappointed in the treatment of phthisis by Koch's tuberculin R., but in the treatment of surgical tuberculosis, such as tuberculous peritonitis, meningitis, bone and joint disease, enlarged glands and lupus, the results with tuberculin R. (Koch) have been most excellent, and I have at present a great many cases under treatment in hospital by this tuberculin as supplied by the Clinical Research Association.

I have recently had prepared from my own cultures of bovine tubercle a tuberculin for use in phthisis pulmonalis with, so far, encouraging results. A perusal of my paper in the April, 1907, number of *Tuberculosis* (Berlin) will explain the reason why tuberculin from bovine sources should be used for phthisis, whilst Koch's tuberculin R., which is prepared from human tubercle bacilli, should be used for the other forms of tuberculosis. The general results and impressions of that work are given here, but in the limited space at my disposal it is impossible to do more than touch generally on a few of the more important points investigated during this research.

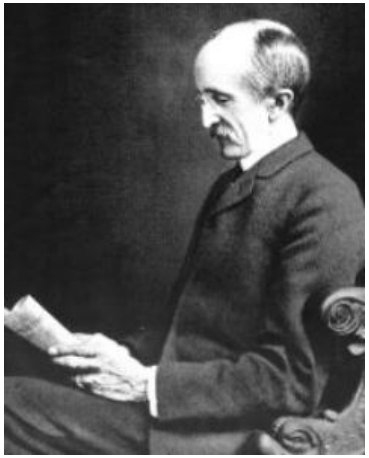
Following the lines of my original paper of 1903, I have no reason to modify the view then set forth—namely: "That human and bovine bacilli are divisible into two distinct it is exactly what we would expect. Consequently I have had prepared from one of my own pure cultures from bovine sources a special tuberculin for the treatment of phthisis pulmonalis. The tuberculin was made from a typical culture of "perlsucht" and was very carefully sterilised and standardised. Several guinea-pigs were inoculated but without any bad effect. Working on these lines I am at present treating over 70 cases of surgical or bovine tuberculosis in the wards of Mill Road infirmary with Koch's tuberculin R., commencing with very small doses and slowly increasing up to a maximum dose of one-hundredth of a milligramme. The results, without any accessory treatment, have been beyond all my anticipation. Enlarged glands, joints, and lupus have been immensely improved, whilst discharging sinuses have cleared up and in two cases the symptoms of tuberculous meningitis associated with tuberculous peritonitis entirely disappeared. The full results of this large number of cases treated by tuberculin will be published in due course. On the other hand, I have treated 16 cases of early phthisis, four of which were associated with hæmoptysis and all of which showed tubercle bacilli in the sputum, with distinctive physical signs at one apex, with the tuberculin prepared from a pure culture of bovine tubercle (kindly supplied to me by Professor Calmette of Lille). These cases are still under treatment, but up to the present many of them have shown marked improvement, with total disappearance of physical signs. It is too soon yet to speak of the final results of treatment, but I hope to publish them after one year. My tuberculin should only be used in early cases and, if possible, in conjunction with open-air or sanatorium treatment. The directions and dosage issued by the Clinical Research Association should be strictly adhered to if good results are to be obtained.

With a view to produce immunity against human tubercle in children, especially in those who have been exposed to infection from a consumptive father or mother, I have lately been working with the serum of tuberculous cattle. I have purchased several dairy cows suffering from tuberculosis of the udder and have obtained, with the kind coöperation of Professor H. E. Annett, a large amount of the serum of these cows. This serum has been very carefully sterilised and injected into guinea-pigs without any ill-effects. I believe that the serum of a cow which has suffered from bovine tuberculosis will confer such immunity when injected into a child as will suffice to protect him against an attack of human tuberculosis. Considering the large number of children who are attacked by phthisis pulmonalis as the direct result of contact with a consumptive parent a protective serum would

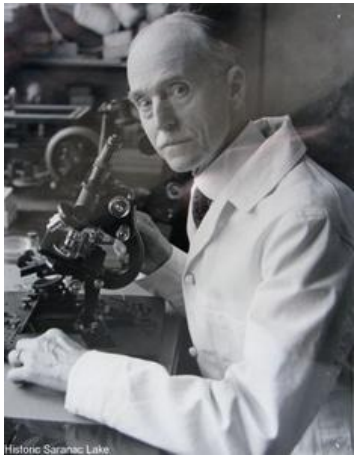
# İlk aşı araştırmaları

- Çağın en önemli bilim adamları tüberküloza aşı için araştırmalar yaptılar.

Louis Pasteur



Edward Trudeau



Edward Robinson Baldwin

Theobald Smith

# 1904 Aşı çalışmaları

- 1904 yılında Albert Calmette'nin sığırdan izole ettiği *M. bovis* izolatı,

  1908 yılında Jean-Marie Camille Fransa, Pasteur enstitüsünde, seri pasajlarını yapmaya başladı.

Albert Calmette

Camille Guérin

# 1921 Oral Formda BCG

- Geliştirilen ilk aşı «oral formda» hazırlanmıştı ve Pariste risk altında yeni doğan bebeklere uygulandı.
- İlk olarak 100.00 bebek aşılandı.

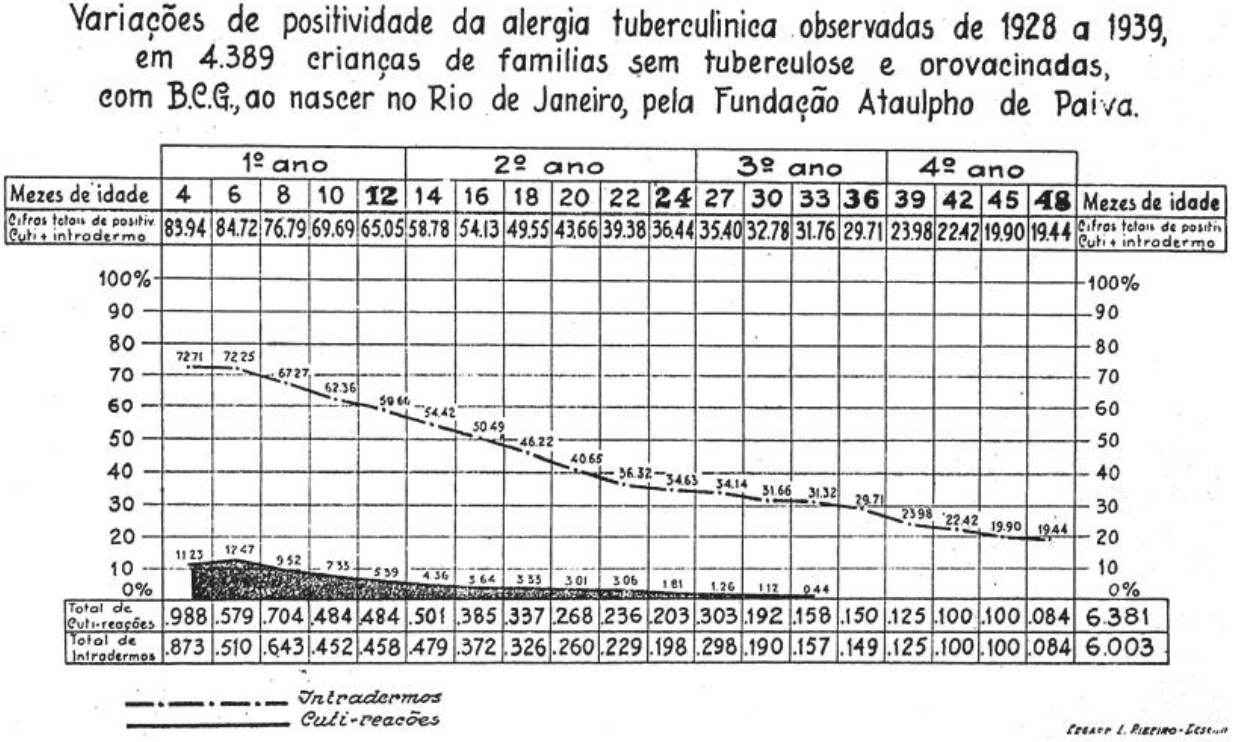
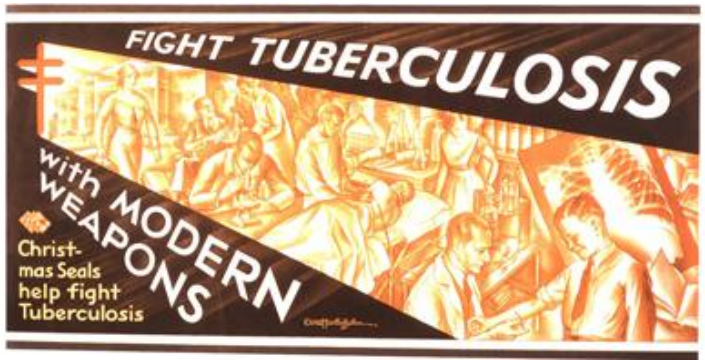



Fig. 3: skin tests in orally immunized neonates – 48 month follow-up. This 1940 graph shows variations on positive skin tests (mean 70% after immunization and 20% four years later) observed in 4389 children belonging to tuberculosis free families and vaccinated with oral BCG on birth, in Rio de Janeiro. This graph is the faithful copy of the original

# 1921 BCG

- Lubeck Felaketi;

  72 bebek, canlı suş ile kontamine oral BCG aşısından hayatını kaybetti.




Science

REPORTS

THE LÜBECK DISASTER[1]

Of the children inoculated in Lübeck with the BCG vaccine, more than fifty have died. Unfortunately, according to medical opinion, further deaths are to be expected, as the disease covers a period of from one to two months and the vaccinations were carried out at different times. The federal ministry of the interior has just published a statement based on the results of the inquiry as far as it has progressed. The statement throws a new light on the events in Lübeck and shows with what energy all persons in authority are working to clear up the matter. The statement of the federal ministry of the interior is expressed in precise terms and reads thus:

As was unfortunately to be expected, the terrible disaster that overtook the population of Lübeck in connection with the treatment to establish in children immunity to tuberculosis has not proved to be a catastrophe of only short duration but a calamity involving a series of fatalities and protracted illnesses the end of which is not yet definitely in sight. It is easily intelligible that the excitement over the sad event does not die down at once and that at home and abroad the demand for a more complete explanation of the disaster continues to persist. From the tone of the statements made by the federal minister of the interior, May 21, at the session of the head committee, and, June 16, at the plenary session of the reichstag, it was plainly evident that the investigations of the matter had been begun promptly and that they would be prosecuted without sparing any person or the prestige of any scientific method. Since, however, in some quarters suspicions to the contrary found expression, attention must be called to the fact that the scientific side of this affair involves some of the most difficult problems of bacteriology. The Federal Health Bureau was entrusted by the Federal Ministry of the Interior with the prosecution of the scientific investigations. The definitive outcome of the inquiry can not be announced before three to four weeks.

So far as it is possible to form an opinion from the investigations to date carried on by Professor Dr. Ludwig Lange, who was entrusted with this end of the research, it may be stated that the Calmette culture supplied by the Pasteur Institute in Paris was above reproach, but that it became contaminated during the process of recultivation in Lübeck. It is not open to question but that the Federal Health Bureau is using all available scientific means in the investigations that are being carried on to throw light on the complicated problem—investigations that are planned on a wide scale and will require the use of 600 or more experi-

[1] Berlin correspondent of the *Journal* of the American Medical Association.

# 1928 BCG

- *M. tuberculosis'e* karşı geliştirilen tek lisanslı aşı olan;

  *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) aşısı 1927 yılından itibaren intradermal olarak uygulanmaktadır.

# Etkili Aşı Geliştirme Çabaları

- Tüberküloza karşı etkili bir aşı geliştirilebilmesi için;

  konak ve patojen arasında gelişen moleküler etkileşimlerin açığa kavuşturulması

  ve antijenik yapıların araştırılması gereklidir.

**Basil** ⟷ **Konak**

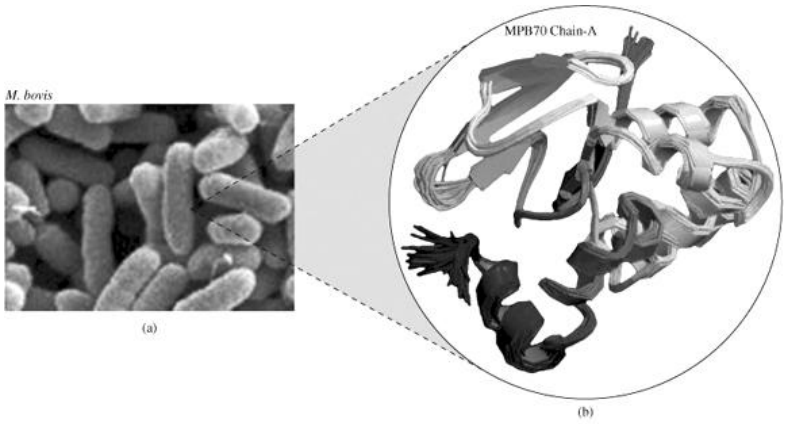



**Figure 1.** *Mycobacterium tuberculosis*; Solution Structure of the Antigenic Tb Protein MPT70/MPB70; Authors: M. J. Bloemink, E. Dentten, R. G. Hewinson, R. A. Williamson, M. D. Carr; Exp. Method: NMR, Polymer Chains: A; Residues: 163; Source: The RCSB Protein Data Bank.

# Mikobakterilerin antijenik komponentlerinin karakterizasyonu

- Mikobakterilerin antijenik komponentlerinin karakterizasyonu,

  mikobakterilere karşı gelişen immün cevabın anlaşılmasında anahtar rol oynar.

INFECTION AND IMMUNITY, Oct. 1972, p. 564–573

Vol. 6, No. 4
*Printed in U.S.A.*

## Characterization and Comparison of Mycobacterial Antigens by Two-Dimensional Immunoelectrophoresis

DIANNA B. ROBERTS,[1] GEORGE L. WRIGHT, JR., LEWIS F. AFFRONTI, AND MELVIN REICH

*Department of Microbiology, The George Washington University School of Medicine, Washington, D.C. 20005*

Received for publication 27 April 1972

# Mikobakterilerin antijenik komponentlerinin karakterizasyonu

- Buna bağlı olarak,

  aşıların yanında tanı testlerinin geliştirilmesi için de hedef antijenik moleküllerin tespit edilmesi ve birbirileriyle karşılaştırılması gereklidir.

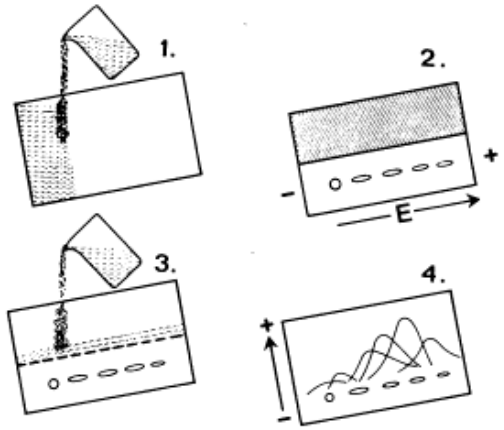



FIG. 1. *Diagrammatic illustration of the procedure for performing two-dimensional immunoelectrophoresis. 1. A hot (90 C) solution of 1% agarose in barbital buffer is poured onto an 8 by 10 cm glass slide. 2. Electrophoresis in the first direction is carried out at 12 ma per slide. The agarose in the region of the diagonal lines is then cut away. 3. A warm (50 C) mixture of equal parts 2% agarose in barbital buffer and antiserum is poured onto the slide to cover the region above the dashed line. 4. The second electrophoresis is then carried out at right angles to the first at 10 ma per slide for 20 hr.*



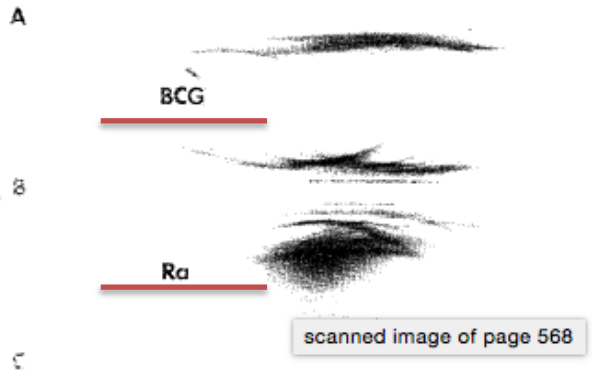



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FIG. 3. *Conventional immunoelectrophoretic patterns of reactions of Mycobacterium bovis strain BCG (BCG) cell extract (CX) and M. tuberculosis strain H37Ra (Ra) CX with rabbit anti-BCG (troughs A and C) and rabbit anti-Ra (trough B).*

# Mikobakterilerin antijenik komponentlerinin karakterizasyonu

- Mikobakteriyel antijenlerin yapısı ve bunlara karşı gelişen immün yanıt ile ilişkili olarak ilk geniş çaplı araştırma 1978 yılında Daniel ve Janicki tarafından yapılmıştır.

MICROBIOLOGICAL REVIEWS, Mar., 1978, p. 84–113
0146-0749/78/0042-0084$02.00/0

Vol. 42, No. 1
*Printed in U.S.A.*

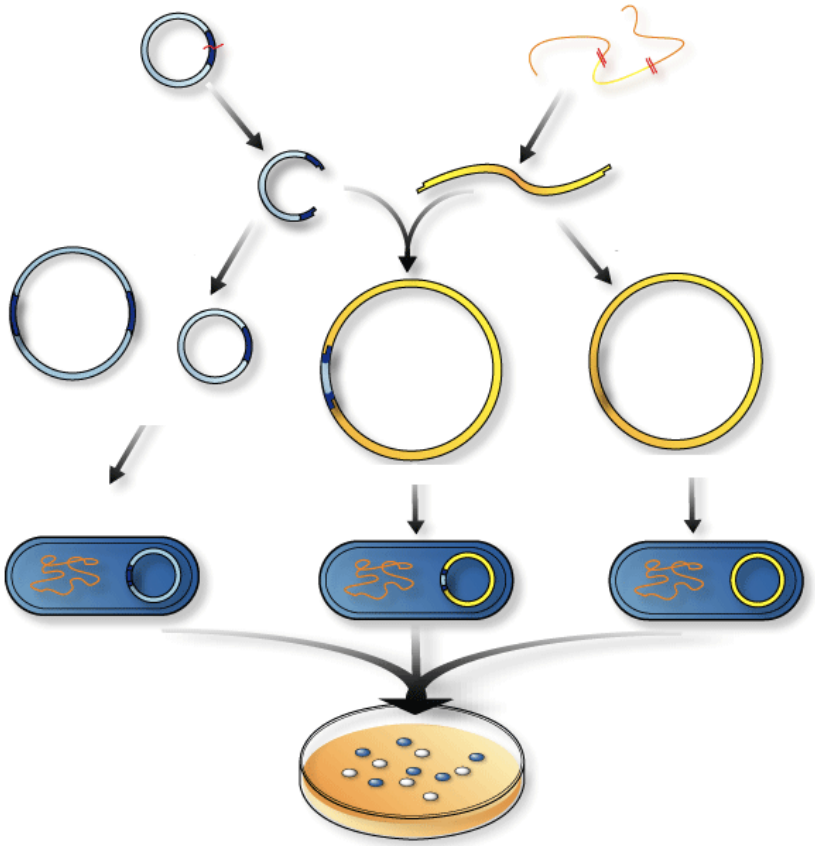
## Mycobacterial Antigens: a Review of Their Isolation, Chemistry, and Immunological Properties

THOMAS M. DANIEL[1]* AND BERNARD W. JANICKI[2]

*Department of Medicine, Case Western Reserve University and University Hospitals, Cleveland, Ohio 44106,[1] and National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20014[2]*

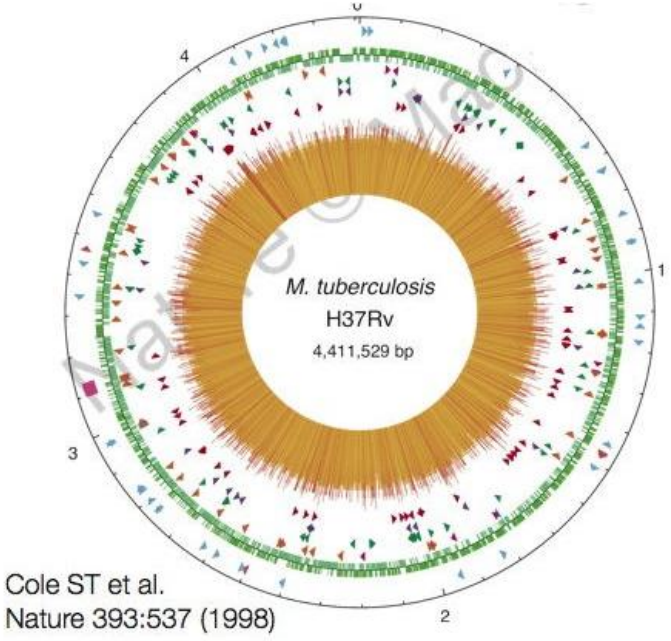
# Mikobakterilerin antijenik komponentlerinin karakterizasyonu

- Bu tarihten sonra klonlama teknolojilerinin geliştirilmesi ile mikobakteriyel antijenlerin kompetan hücrelerde ekspresyonları ve antijenlerin saf olarak eldesi başarılmıştır.

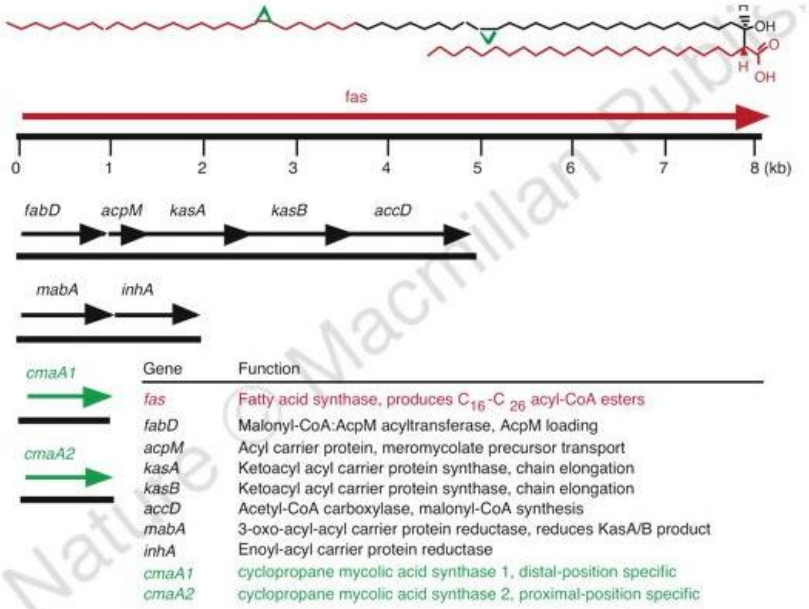


# Mikobakterilerin antijenik komponentlerinin karakterizasyonu

- Bu alana katkıda bulunan ikinci önemli gelişme ise dizi analizi yöntemlerindeki ilerlemelerdir.




Cole ST et al.
Nature 393:537 (1998)

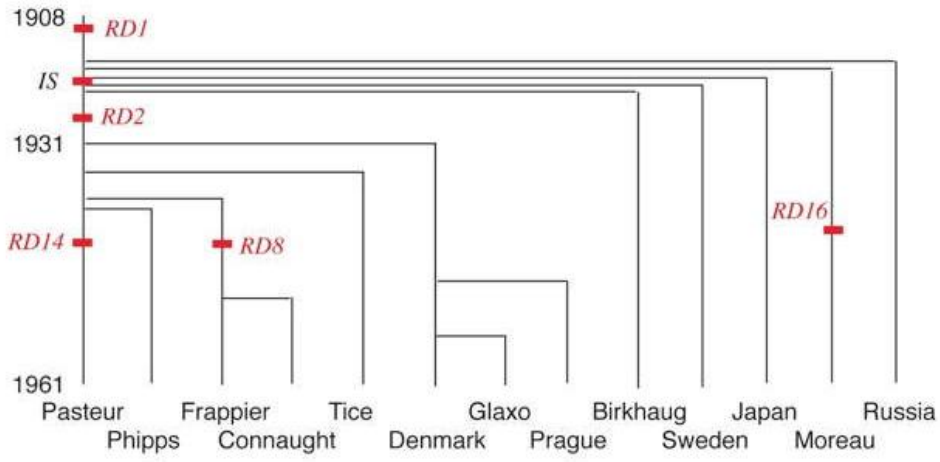


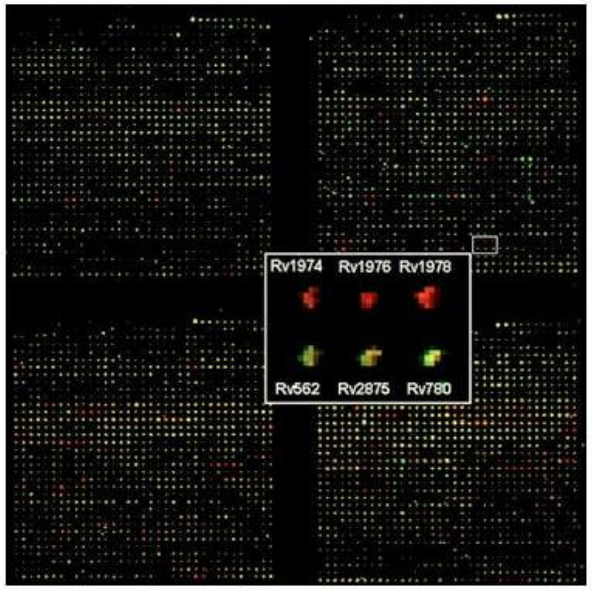

| Gene | Function |
|---|---|
| *fas* | Fatty acid synthase, produces $C_{16}$-$C_{26}$ acyl-CoA esters |
| *fabD* | Malonyl-CoA:AcpM acyltransferase, AcpM loading |
| *acpM* | Acyl carrier protein, meromycolate precursor transport |
| *kasA* | Ketoacyl acyl carrier protein synthase, chain elongation |
| *kasB* | Ketoacyl acyl carrier protein synthase, chain elongation |
| *accD* | Acetyl-CoA carboxylase, malonyl-CoA synthesis |
| *mabA* | 3-oxo-acyl-acyl carrier protein reductase, reduces KasA/B product |
| *inhA* | Enoyl-acyl carrier protein reductase |
| *cmaA1* | cyclopropane mycolic acid synthase 1, distal-position specific |
| *cmaA2* | cyclopropane mycolic acid synthase 2, proximal-position specific |

# Mikobakterilerin antijenik komponentlerinin karakterizasyonu

- Peptid kütüphanelerinin ve protein mikroarrayleri içeren yeni proteomik yaklaşımların geliştirilmesi, immün dominant antijenlerin ve potansiyel aşı adaylarının araştırılmasını sağladı.

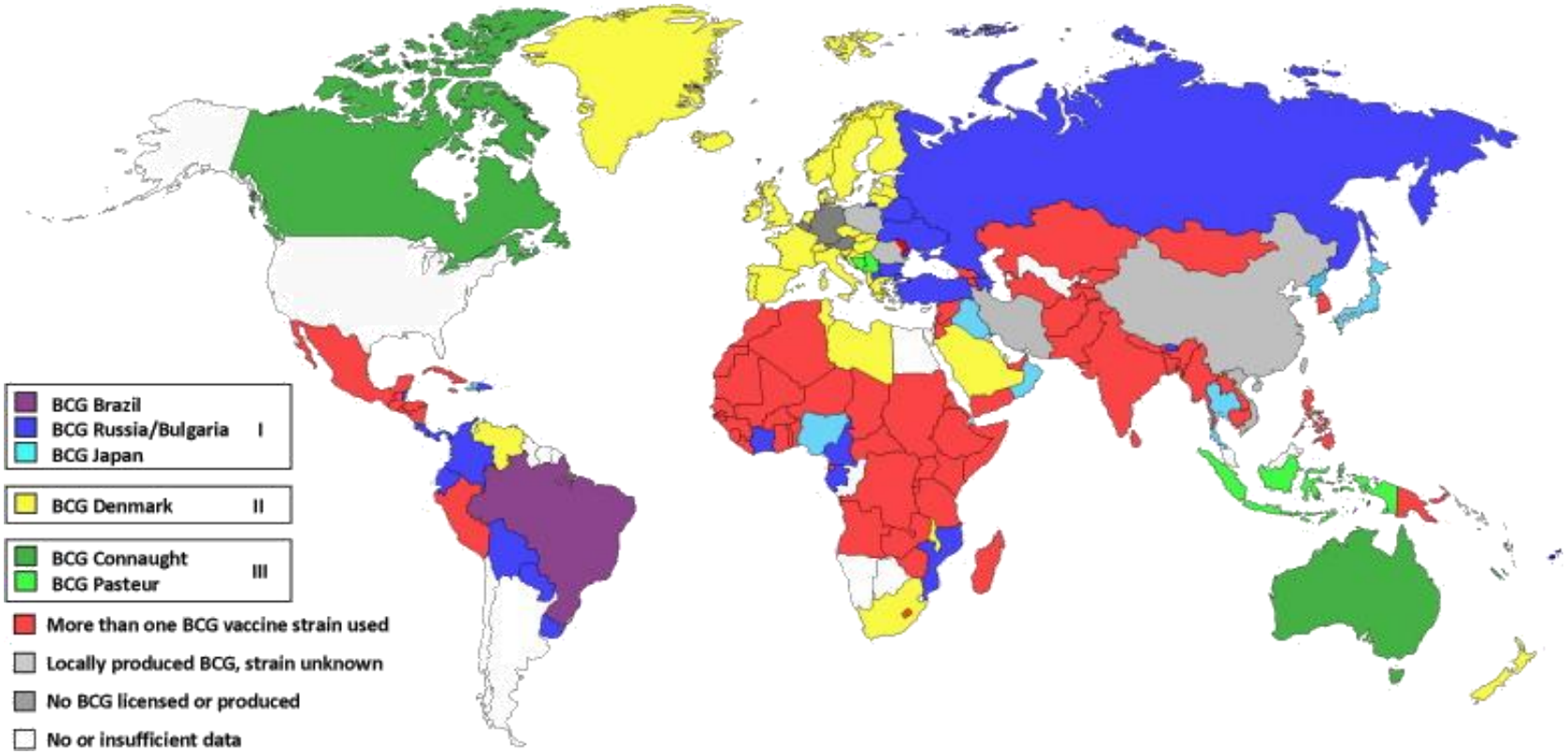
  - *M. tuberculosis* H37Rv suşunda yer alan 11 gen bölgesi (38 ORF içeren) *M. bovis* te yok
  - *M. bovis* te bulunan 5 gen bölgesi (38 ORF içeren), BCG suşunda yok




Behr MA et al., Science 284: 1520 (1999)

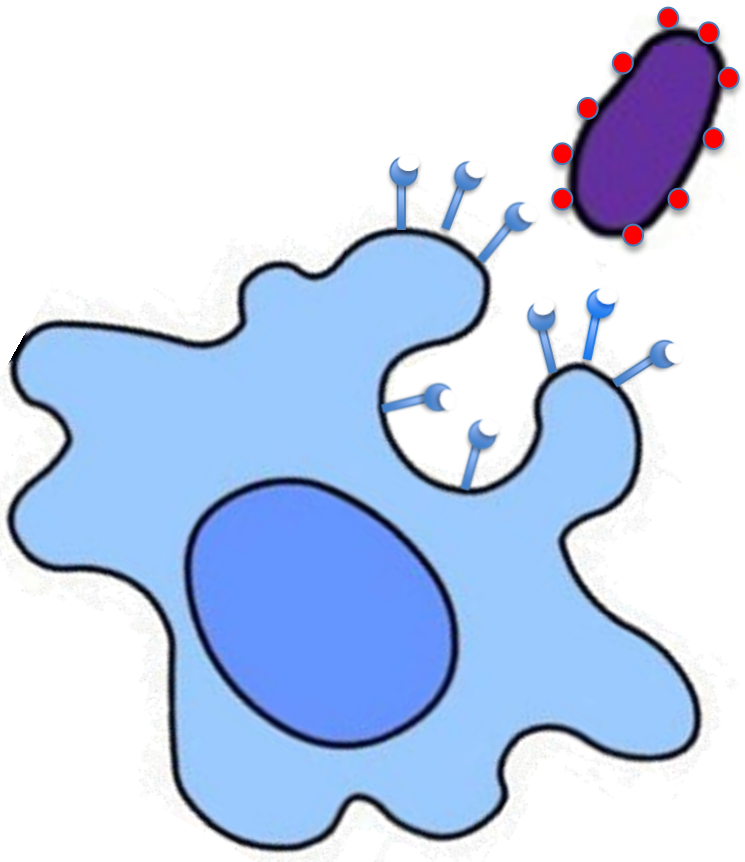
# Mikobakterilerin antijenik komponentlerinin karakterizasyonu




Tuberculosis. 2009 Jul;89(4):248-51.

# Doğal Bağışıklık Mekanizmaları

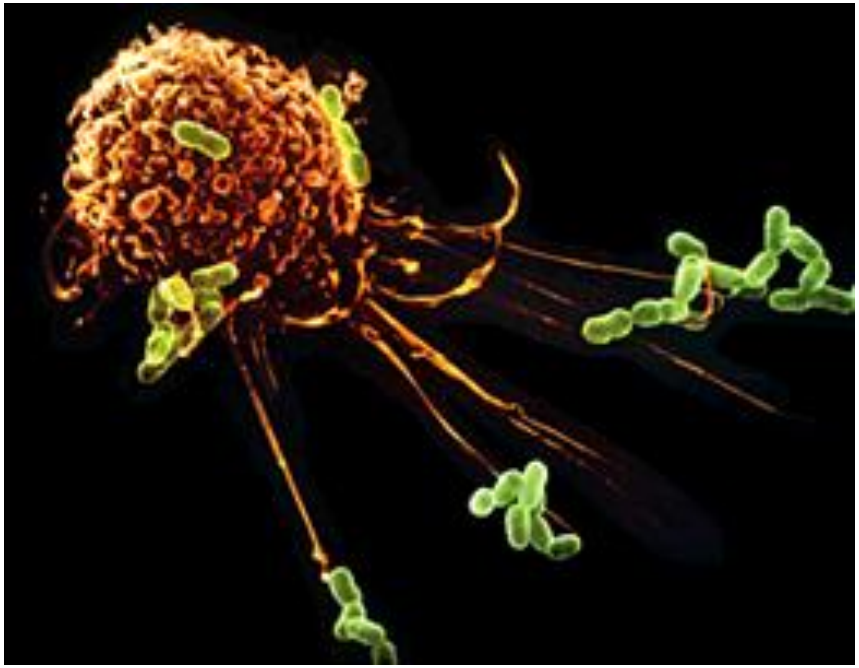
- Doğal bağışıklığın parçaları memeli hücrelerinde bulunmayan fakat mikrobiyal patojenlerde karakteristik olan yapıları tanır.



- Pathogen Associated Molecular Patterns-(PAMP)
  - Mikolik asit
  - Lipomannan (LM)
  - fosfalidil-myo-innositol mannozid (PİM)
  - lipoarabinomannan (LAM) - Arabinogalaktan
- Pathogen Recognition Receptors-(PRR)
  - Toll-benzeri reseptörler (TLRs)
  - nükleotid bağlayan oligomerizasyon boğumu (NOD)
  - benzeri reseptörler (NLRs)
  - C-tip lektinleri (mannoz reseptörleri )
  - dendritic cell-specific intercellular adhesion molecule grabbing nonintegrin-(DC-SIGN) ve
  - Dectin-1 reseptörleri

# Doğal Bağışıklık Mekanizmaları

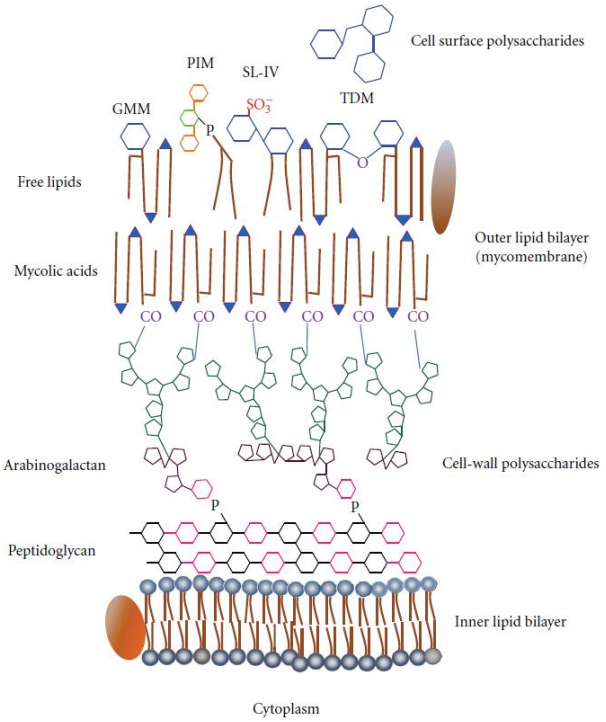
- *M. tuberculosis* ilk olarak akciğerlerde yerleşik olan alveolar makrofajlar ve dendritik hücrelerce temas eder.



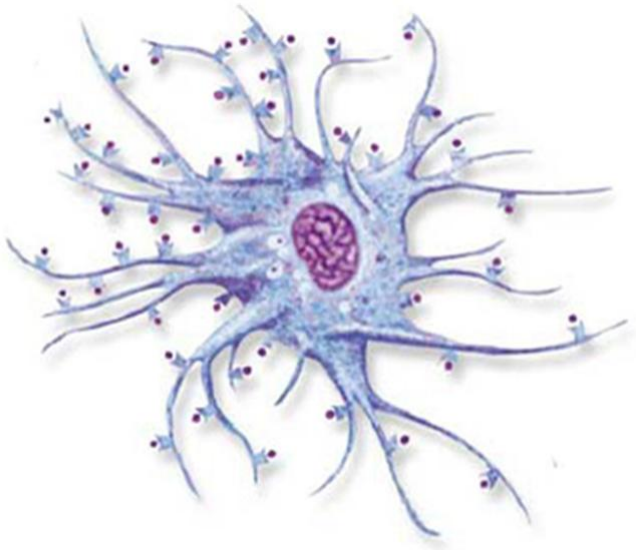
# Doğal Bağışıklık Mekanizmaları

- *M. tuberculosis* ilk olarak akciğerlerde yerleşik olan alveolar makrofajlar ve dendritik hücrelerce temas eder.

Hangi PAMP ?



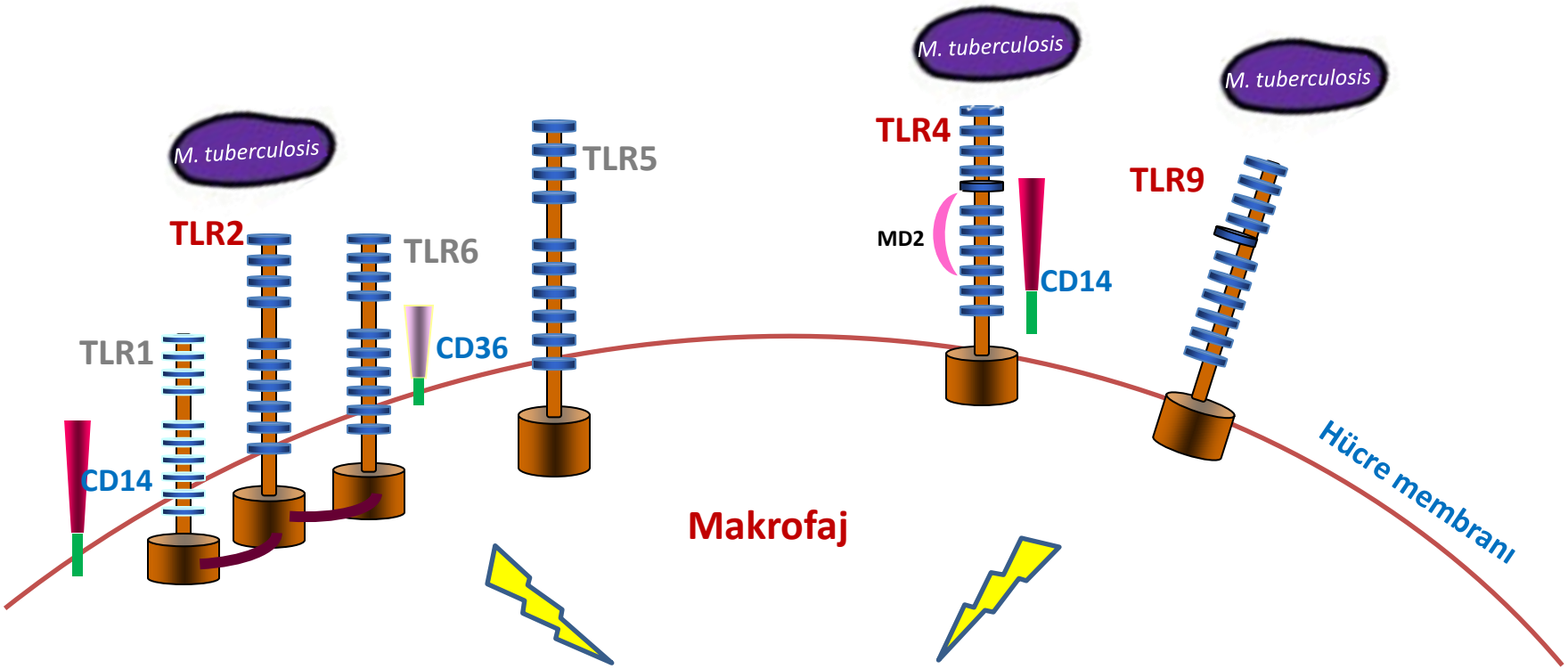

Hangi PRP ?



# Doğal Bağışıklık Mekanizmaları

- *M.tuberculosis TLR'ler ile ilişkisi;*

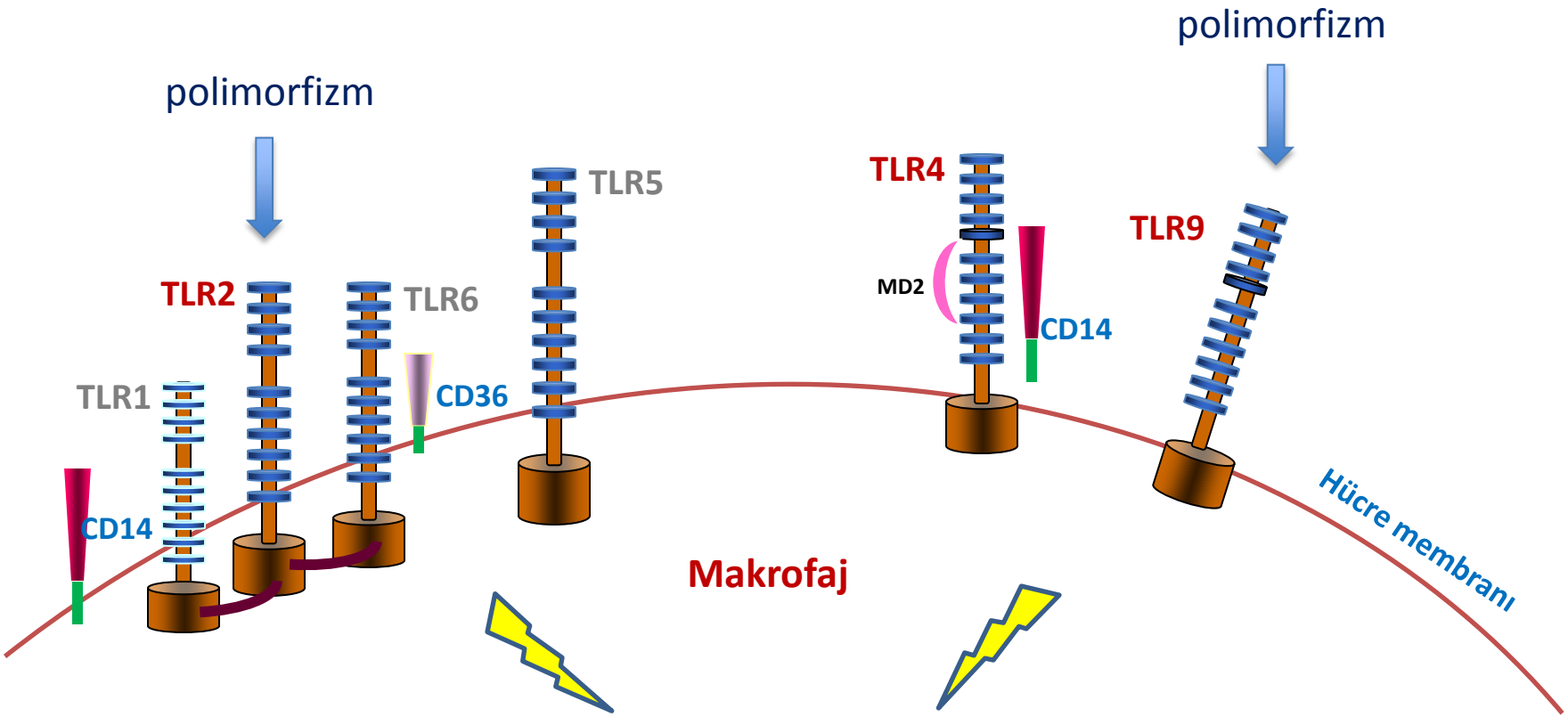
  *konak için yararlı olan proinflamatuar yanıtı başlatan hücre içi sinyal yolaklarını harekete geçirir.*



# Doğal Bağışıklık Mekanizmaları

- TLR'lerdeki gen polimorfizmleri;

  mikobakteriyel lipopeptidlere karşı doğal immün yanıtı düzenler; bu da patojene karşı klinik duyarlılığın nedenlerinden bir tanesidir



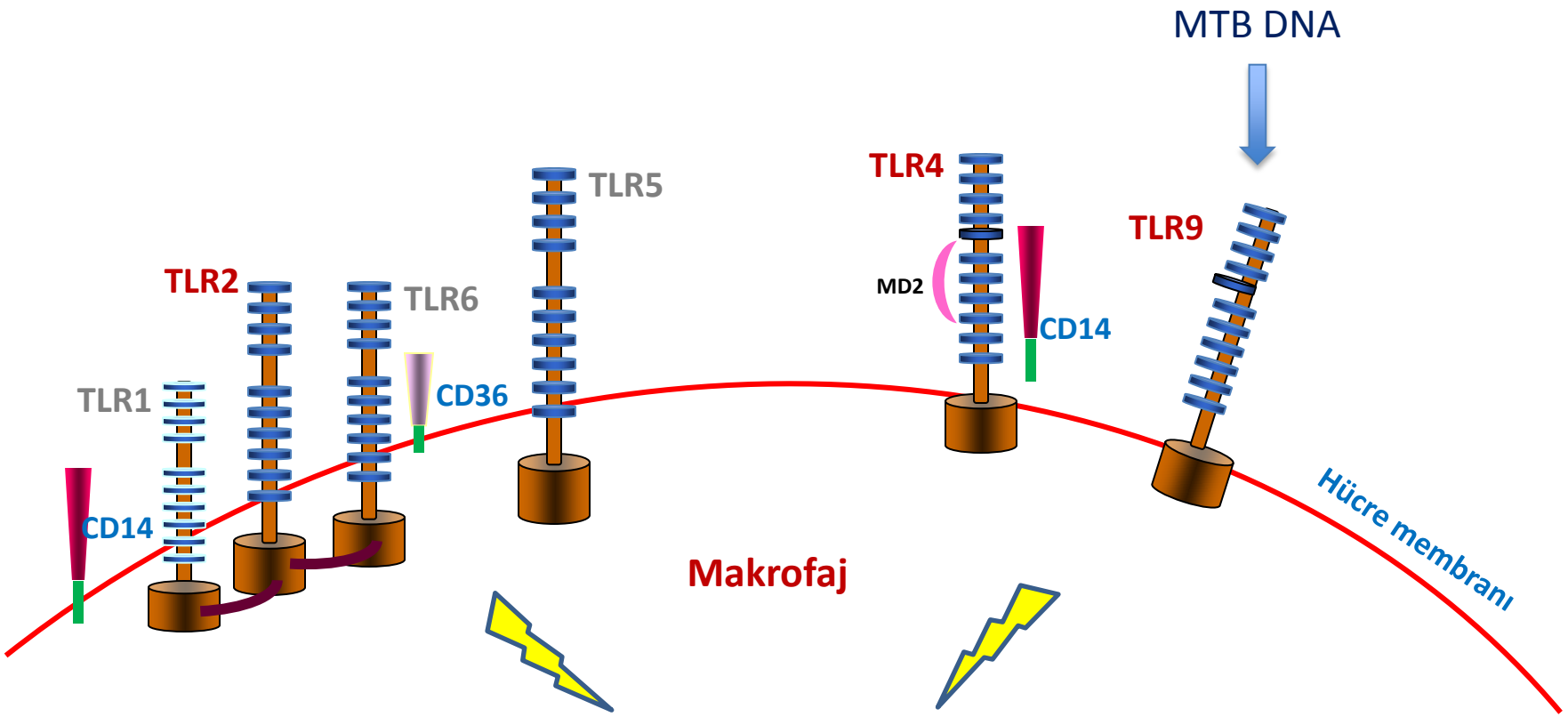
# Doğal Bağışıklık Mekanizmaları

- *M.tuberculosis* in GC-zengin genomu TLR9 ile bağlanmaya uygundur.

  Bu uyarım,

  granülom oluşumunu düzene sokmakta

  T lenfosit-1 (Th1) ve T lenfosit-2 (Th2) sitokin düzeylerini düzenlemektedir.

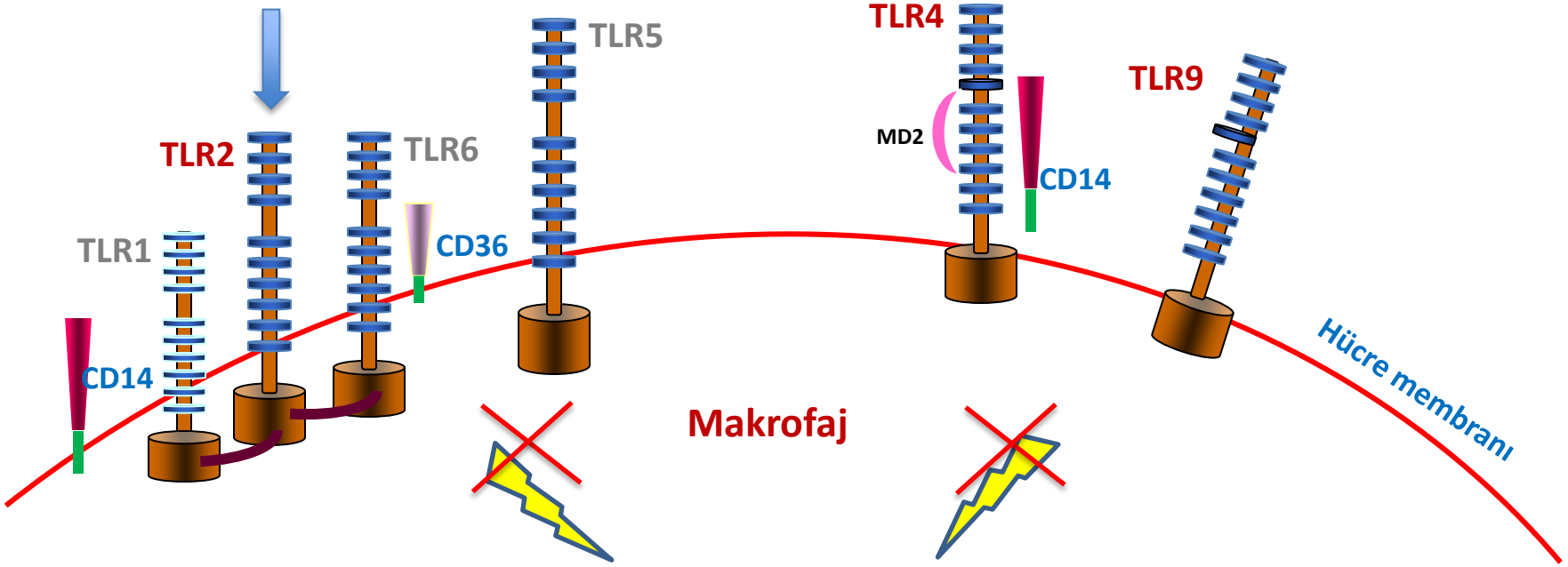


# Doğal Bağışıklık Mekanizmaları

- Bakteri doğal immün yanıtı baskılayan veya düzenleyen

  sinyalleri harekete geçiren yöntemler geliştirmiştir.

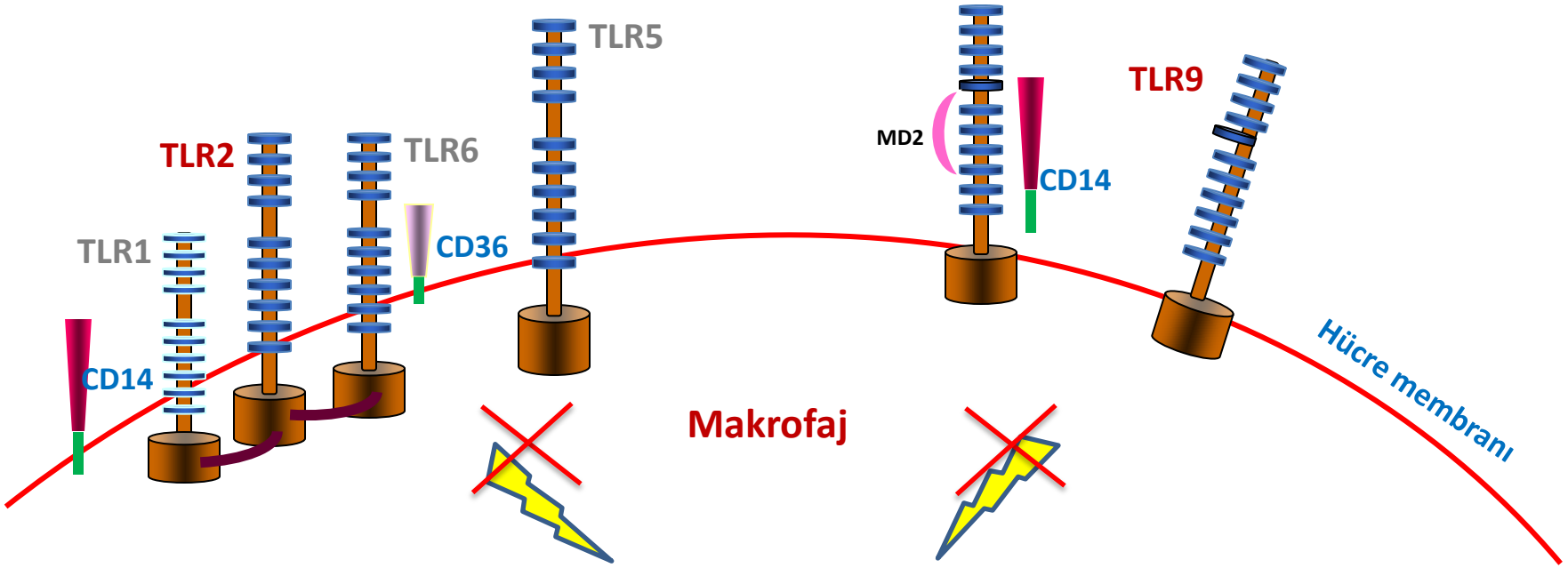
  - sitokin sentezinin inhibisyonu
  - makrofajlarda MHC-sınıf II'nin ifade edilme düzeyini azalması



# Doğal Bağışıklık Mekanizmaları

- Doğal bağışıklık reseptörleri,

  adjuvan görevi yapacak moleküller için iyi hedeflerdir.



# Doğal Bağışıklık Mekanizmaları

- TLR4 agonistleri;

  sentetik monophosphoryl lipid A komibinasyonları adjuvan olarak kullanılmaktadır.

The Importan
of a Tubercul

**A Defined Tuberculosis Vaccine Candidate Boosts BCG and Protects Against Multidrug Resistant *Mycobacterium tuberculosis***

Sylvie Bertholet[1,†], Gregory C. Ireton[1], Diane J. Ordway[2], Hillarie Plessner Windish[1], Samuel O. Pine[1], Maria Kahn[1], Tony Phan[1], Ian M. Orme[2], Thomas S. Vedvick[1], Susan L. Baldwin[1], Rhea N. Coler[1,*], and Steven G. Reed[1,*]

**FIGURE 1.** Increased ID93-specific IgG2c endpoint titers are induced after mouse immunization with ID93/GLA-SE; increased IgG1 titers are induced with ID93/SE. Serum was collected 2 wk after the first immunization (day 14) or 2 wk after the last immunization (day 56). Mean reciprocal dilutions are represented as the endpoint titer ($log_{10}$) + SD. This is one representative study of at least two independent studies. A two-tailed Mann–Whitney *t* test was used to determine significance. $*p < 0.05$ (comparing ID93/SE with ID93/GLA-SE).

ID93

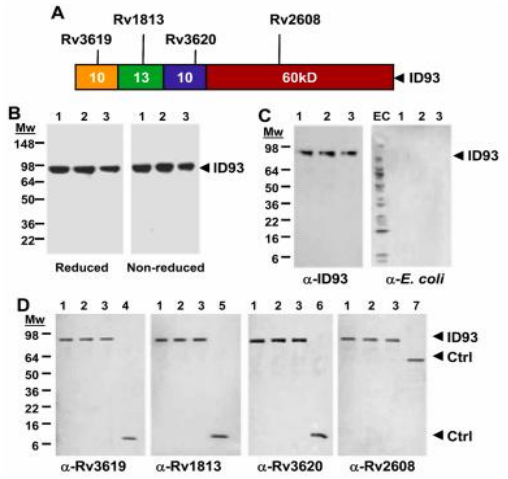
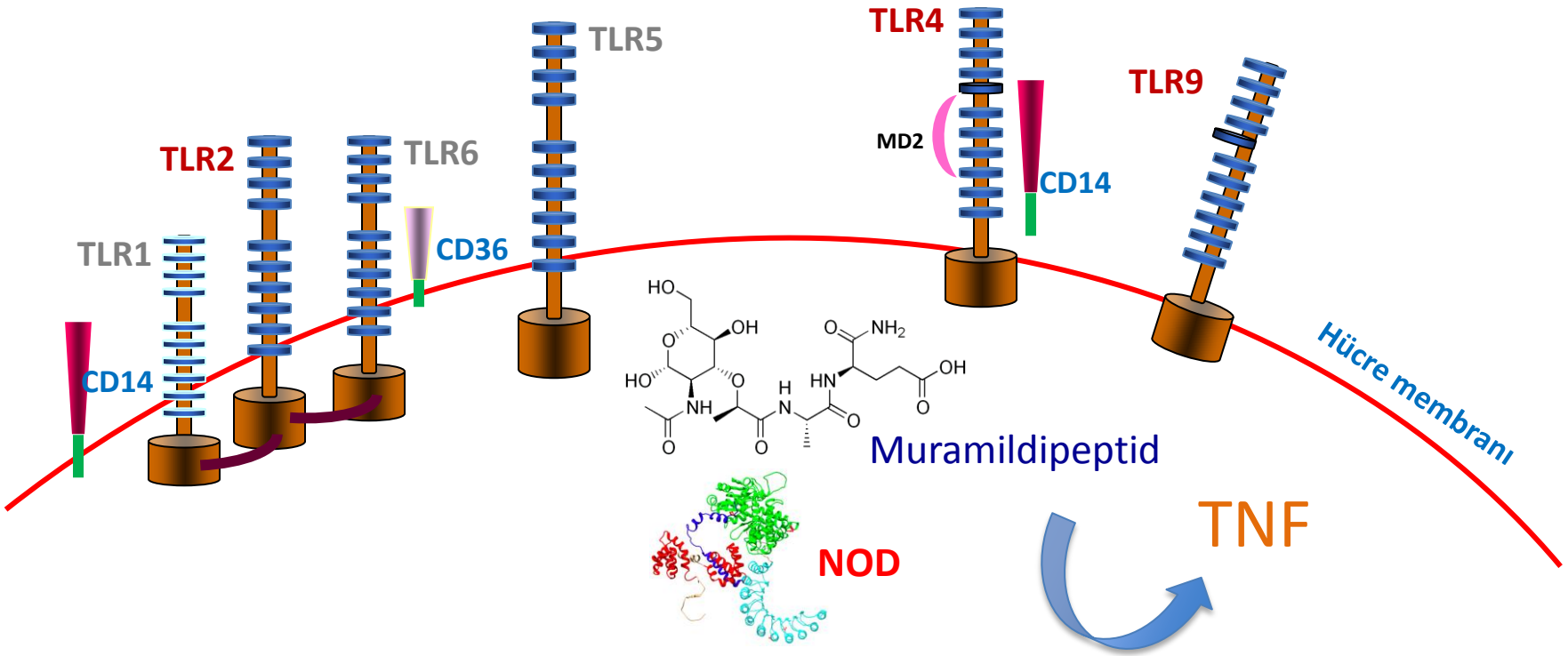



**Fig. 1.**
ID93 protein construct and characterization. **(A)** Schematic of the ID93 fusion protein. **(B – D)** SDS-PAGE and immunoblot of three lots of ID93. **(B)** 2 μg per lane of ID93 (lanes 1 – 3) were run in reducing and non-reducing conditions on a 4 – 20 % Tris glycine gel. **(C)** Immunoblot of ID93 with mouse antibody to ID93 and rabbit anti-sera to *E. coli* (50 ng and 1 μg of ID93, respectively). **(D)** Immunoblot of ID93 with mouse antibody to Rv3619, Rv1813, Rv3620, and Rv2608 (50 ng of ID93). EC, *E. coli* protein standards; ID93, lanes 1 – 3; lane 4, Rv3619; lane 5, Rv1813; lane 6, Rv3620; lane 7, Rv2608.

# Doğal Bağışıklık Mekanizmaları

- Konak hücre içerisinde lösinden zengin tekrar eden proteinler olan

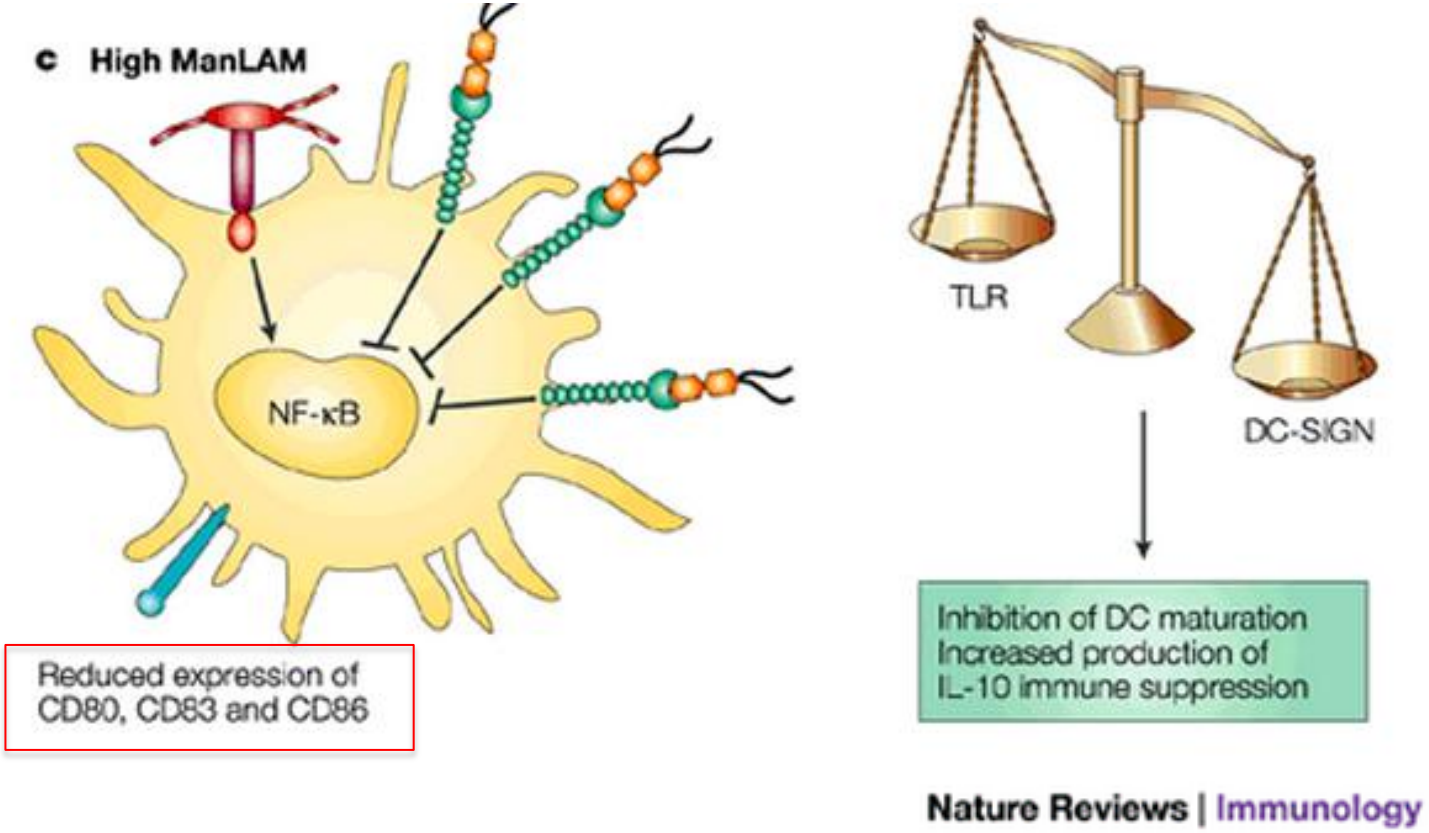
  NOD proteinleri PAMP' lara sitozolde bağlanır.



# Doğal Bağışıklık Mekanizmaları

- Mikobakteriyel lipopolisakkaritler;

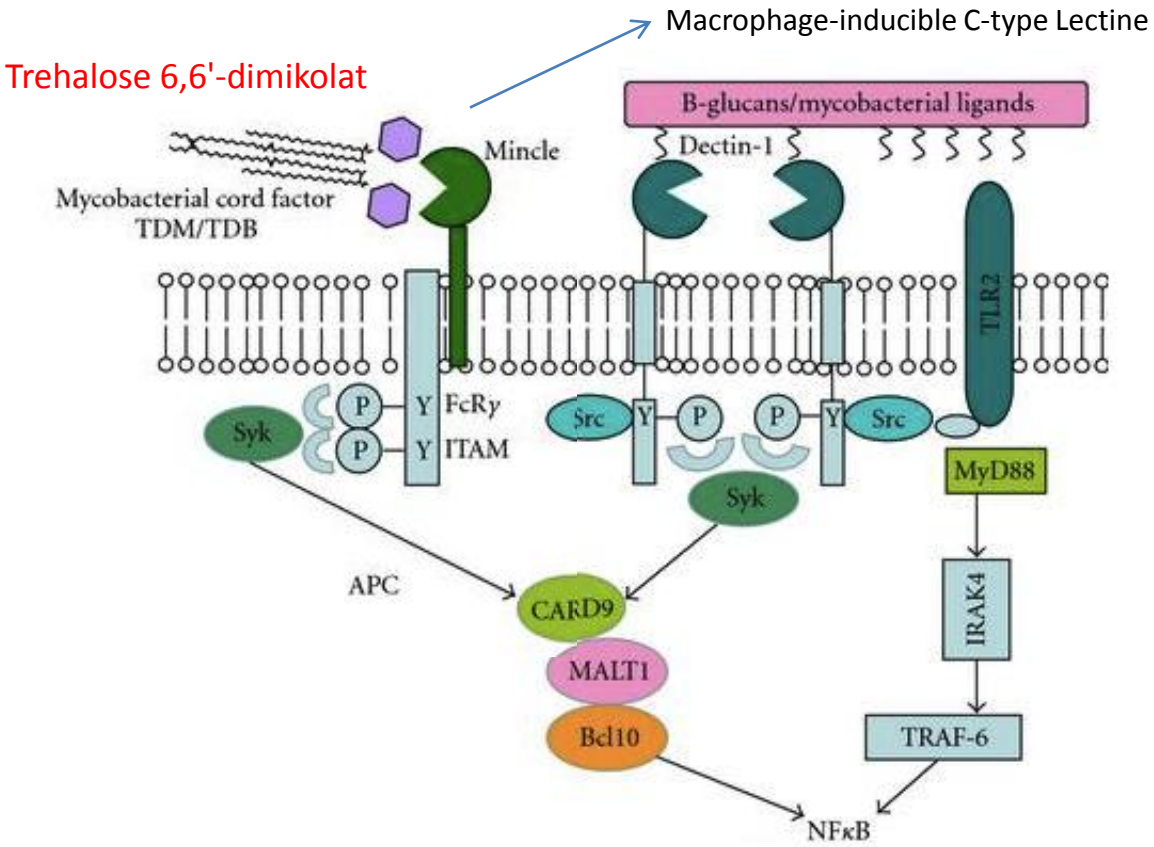
  makrofajların enfekte edilmesinde, bakterilerin hücre içine alınmasında, fagozom gelişiminin engellenmesinde ve anti-inflamatuar yanıtın arttırılmasında önemli rolleri olduğu düşünülmektedir.



# Doğal Bağışıklık Mekanizmaları

- *M.tuberculosis'*in glikolipidyapılarını

  tanıyarak proinflamatuar sinyallerin oluşumunu başlatan diğer bir reseptör Dectin-1'dir.




TNF-a, IL-6, IL-1b, IL-12, IL23 ve IL-17A

# Doğal Bağışıklık Mekanizmaları

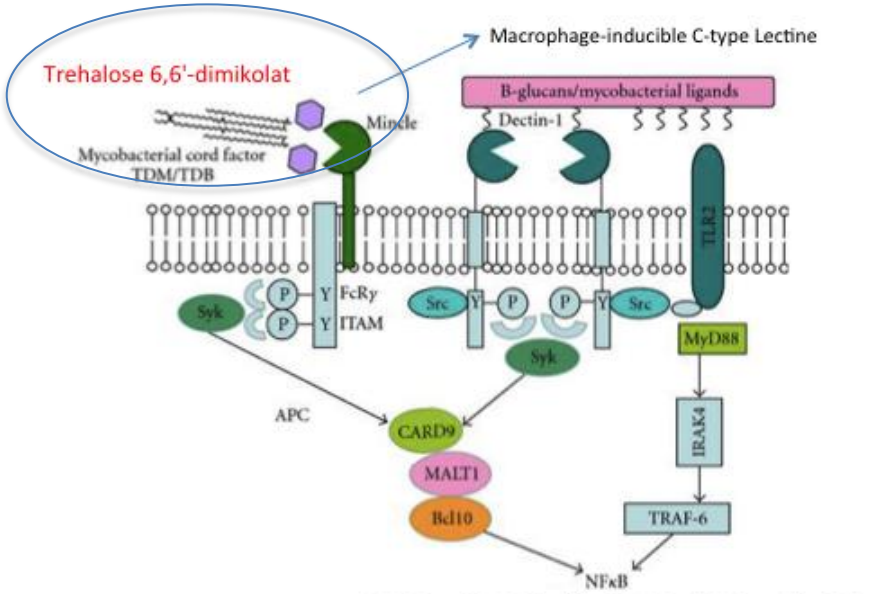
OPEN ACCESS Freely available online

PLOS ONE

## The Mincle-Activating Adjuvant TDB Induces MyD88-Dependent Th1 and Th17 Responses through IL-1R Signaling

**Christiane Desel[1]*, Kerstin Werninghaus[1], Manuel Ritter[2], Katrin Jozefowski[1], Jens Wenzel[1], Norman Russkamp[1], Ulrike Schleicher[1], Dennis Christensen[3], Stefan Wirtz[4], Carsten Kirschning[5], Else Marie Agger[3], Clarissa Prazeres da Costa[2], Roland Lang[1]***

1 Institute of Clinical Microbiology, Immunology and Hygiene, University Hospital Erlangen, Erlangen, Germany, 2 Institute of Medical Microbiology, Immunology and Hygiene, Technische Universität München, Munich, Germany, 3 Statens Serum Institut, Department of Infectious Disease Immunology, Copenhagen, Denmark, 4 Medical Clinic 1, Gastroenterology, Pneumology and Endocrinology, University Hospital Erlangen, Erlangen, Germany, 5 Institut für Medizinische Mikrobiologie, Essen, Germany




TNF-a, IL-6, IL-1b, IL-12, IL23 ve IL-17A

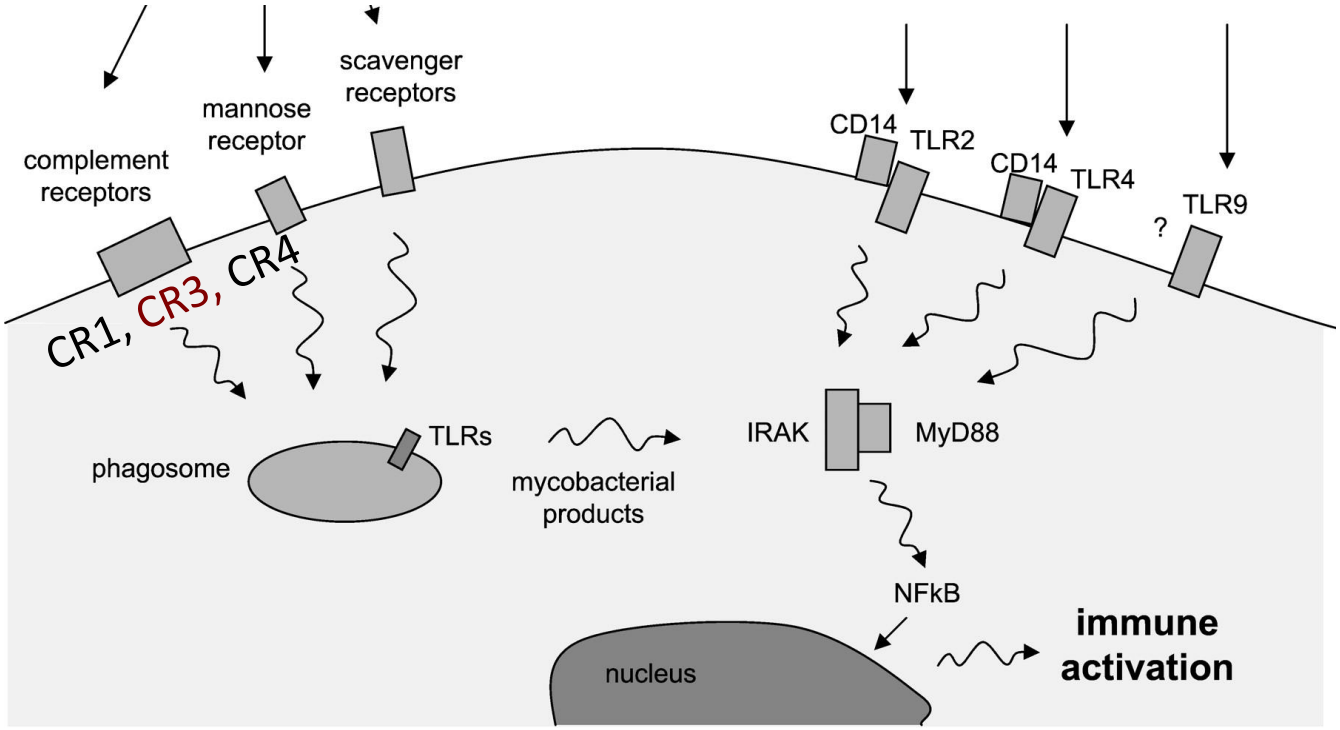
Analog; Trehalose-6,6- dibehenate (TDB)

Th1 ve Th17 indükisyonunda artış

# Doğal Bağışıklık Mekanizmaları

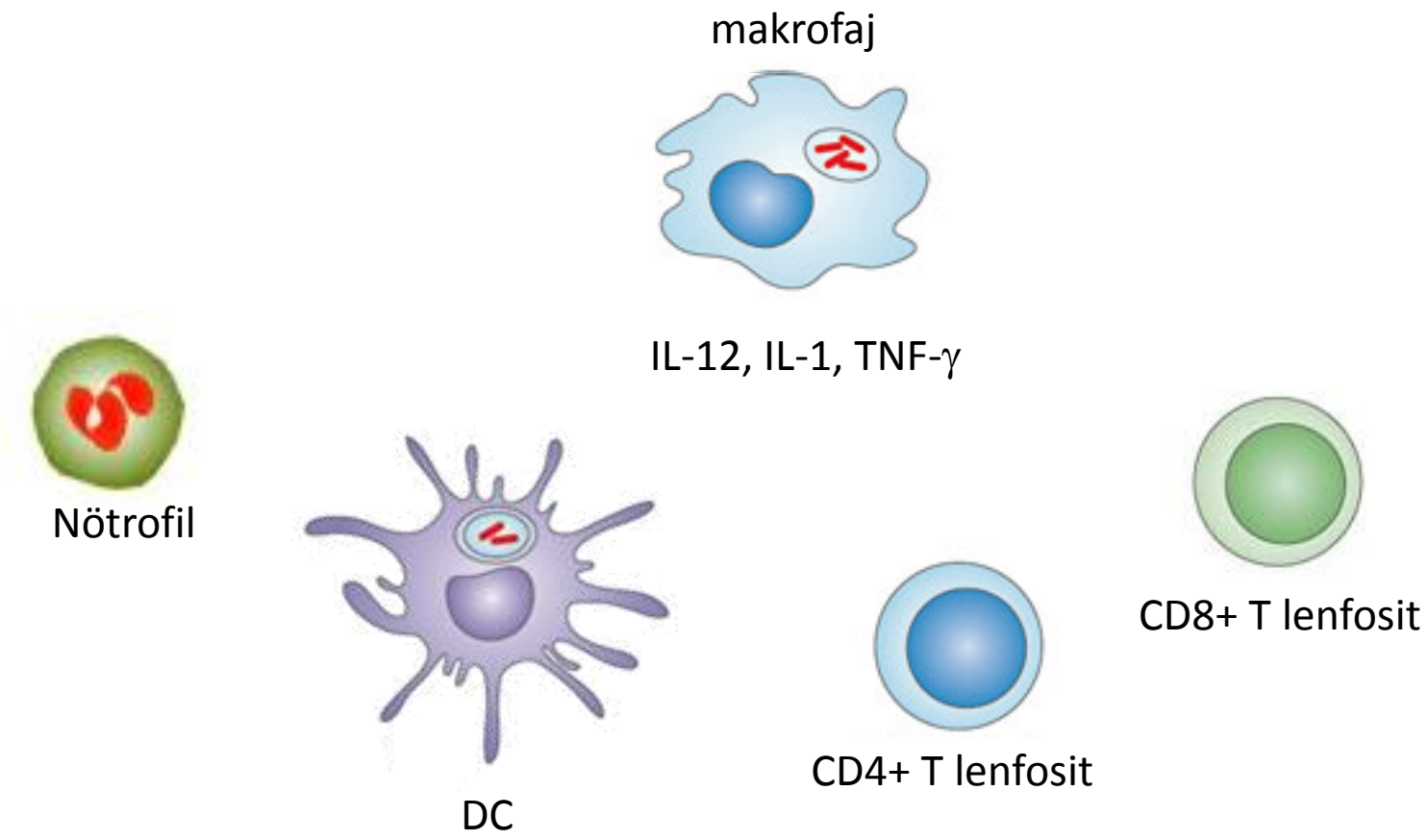
- Tüberküloza karsı immün cevapta

  myeloid differantiation protein 88 (MyD88) kesinlikle gereklidir.



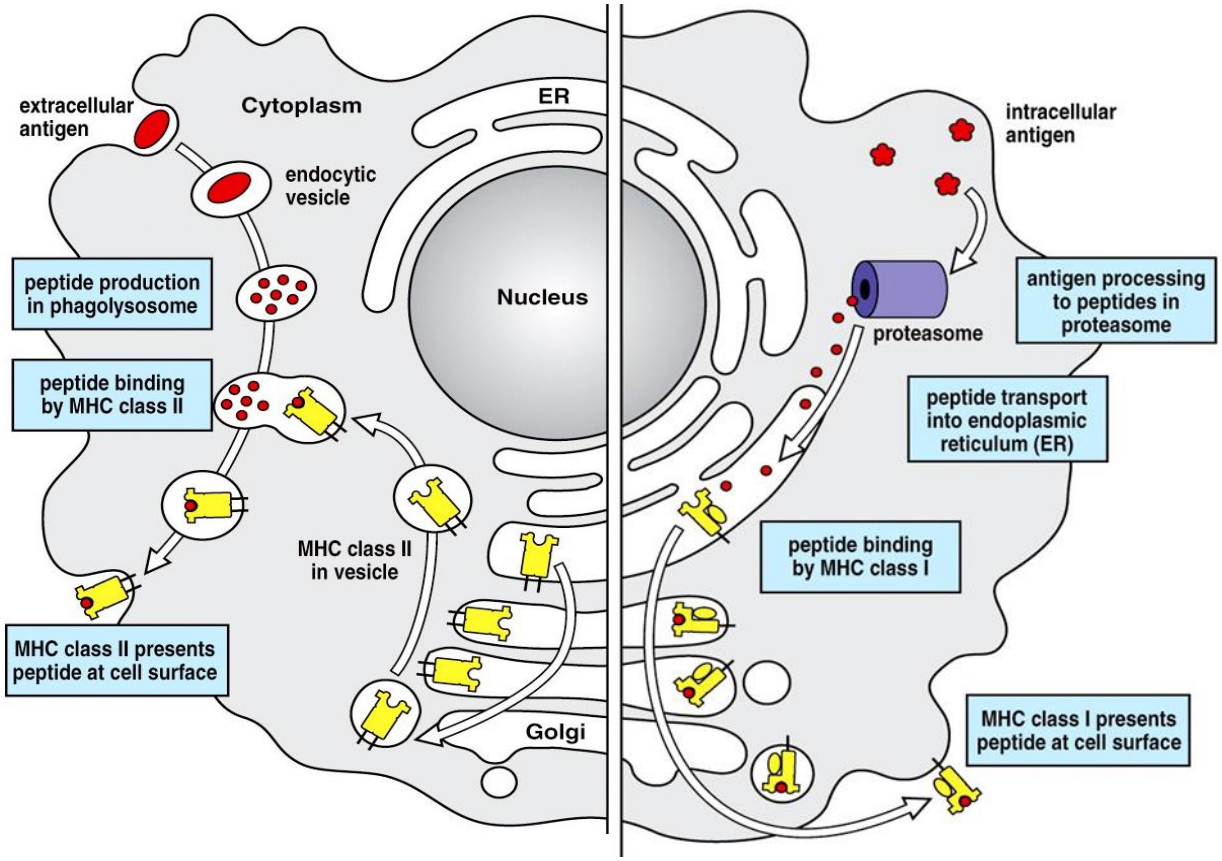
# Kazanılmış Bağışıklık Mekanizmaları

- *M.tuberculosis*'in alveolar makrofajlar içerisine girmesinin ardından, makrofajlarca üretilen proinflamatuar sitokinler ve kemokinler enfeksiyon sinyali olarak görev yapar.



# Kazanılmış Bağışıklık Mekanizmaları

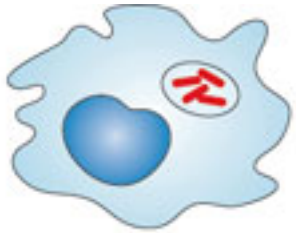
- Aslında olması gereken



# Kazanılmış Bağışıklık Mekanizmaları

- Gerçekte olan
- Basil fagozomal kompartmanların içerisinde;
  - fagozom lizozom inhibisyonunu
  - ATPase ve GTPase'ların birikmesini engelleyebilmekte
  - triptofanaspartam içeren kaplayıcı proteinin fagozomda azaltılmasını
  - tekrarlayıcı glisinden zengin protomin ailesinin üyelerini salgılamayı saglayarak rol oynar.

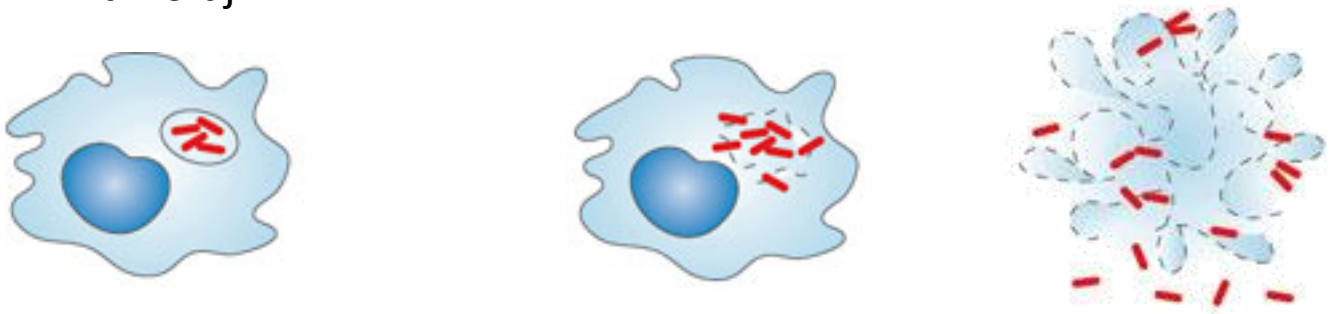
makrofaj



# Kazanılmış Bağışıklık Mekanizmaları

- *M. tuberculosis'in patogenezdeki başarısı, hücrede fagozomal olgunlaşmayı engelleyebilmesi ve fagozomlar içerisinde yaşayabilmesine bağlıdır.*

makrofaj

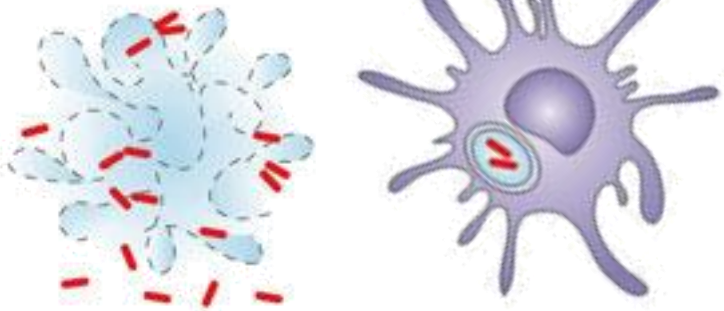


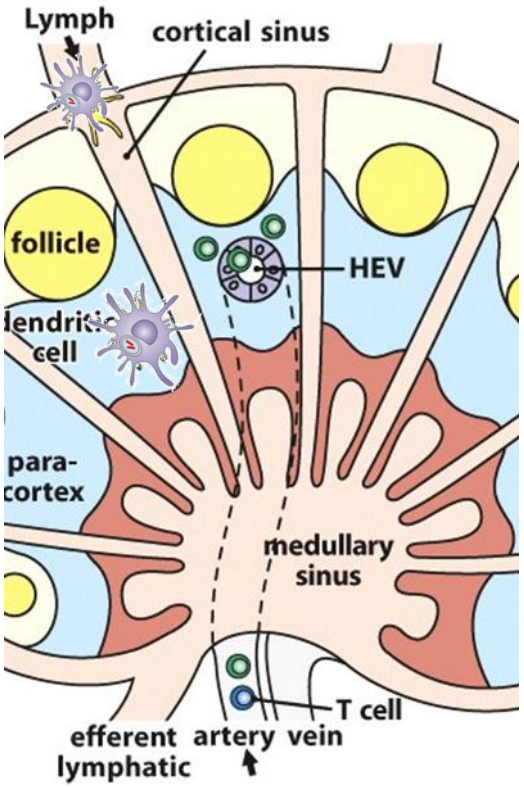
# Kazanılmış Bağışıklık Mekanizmaları

- Bu esnada profesyonel antijen sunucu hücre;

  T lenfositleri (hem CD4 hem de CD8) aktive etmek için, içerisine aldığı basil ile bölgesel lenf düğümlerine doğru hareket eder.

makrofaj

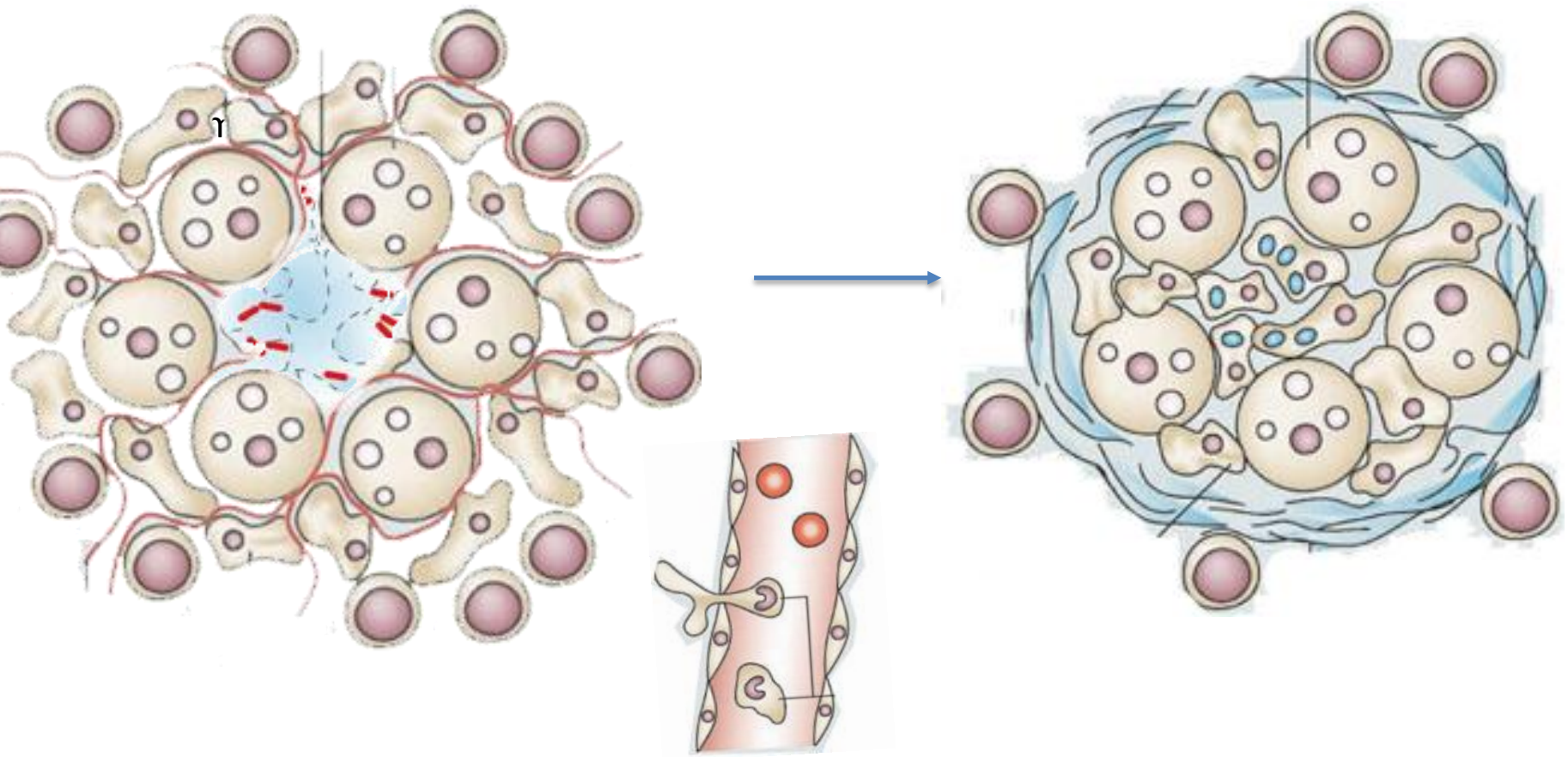




# Kazanılmış Bağışıklık Mekanizmaları

- Aktive T lenfositlerin, enfeksiyon bölgesine hareketi sonucu;

  bu bölgede makrofajlar, T-lenfositleri, dendritik hücreler, fibroblastlar, endothelial hücreler ve stromal hücrelerin birikimi ile oluşan granülom oluşumu gözlenir.



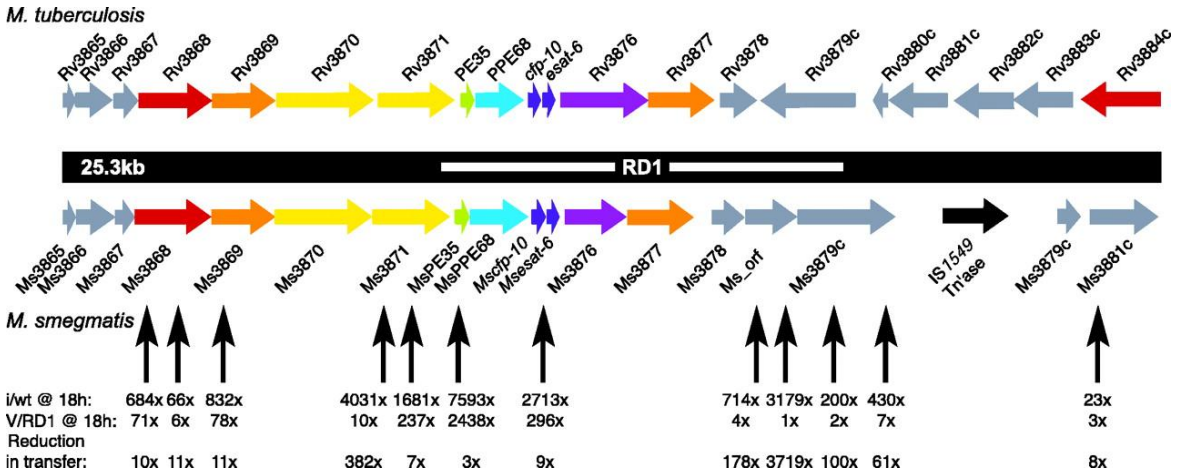
# Kazanılmış Bağışıklık Mekanizmaları

# *Mycobacterium tuberculosis*'in patogenezdeki başarısı ve antijenler

- Mikobakterilerin RD1 lokusları,

  virulansı arttıran özellesmis gizleme sistemini kodlamaktadır.

- RD1'den yoksun bakterinin enfekte içinde üreyebilme yetenegine ragmen etkin bir granülom yapamadıgı saptanmıstır.

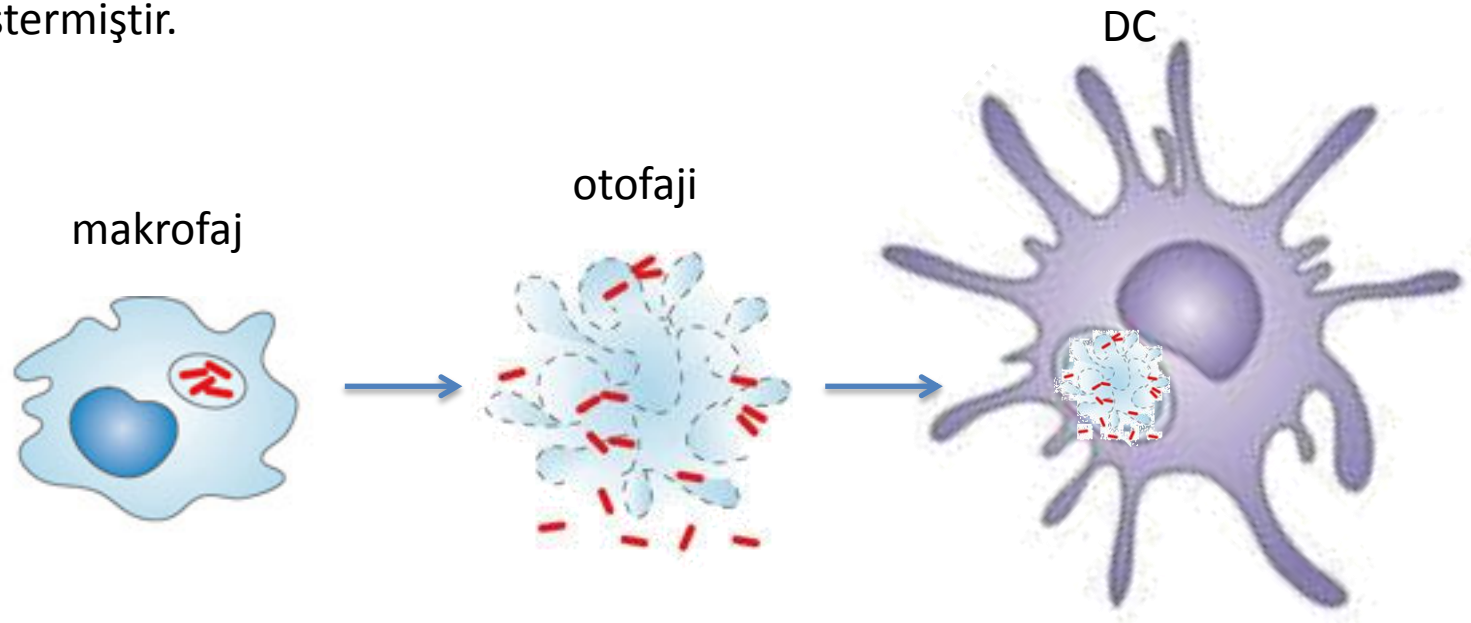



Alignment of the M. tuberculosis and M. smegmatis RD1 regions.

**Jessica L. Flint et al. PNAS 2004;101:12598-12603**

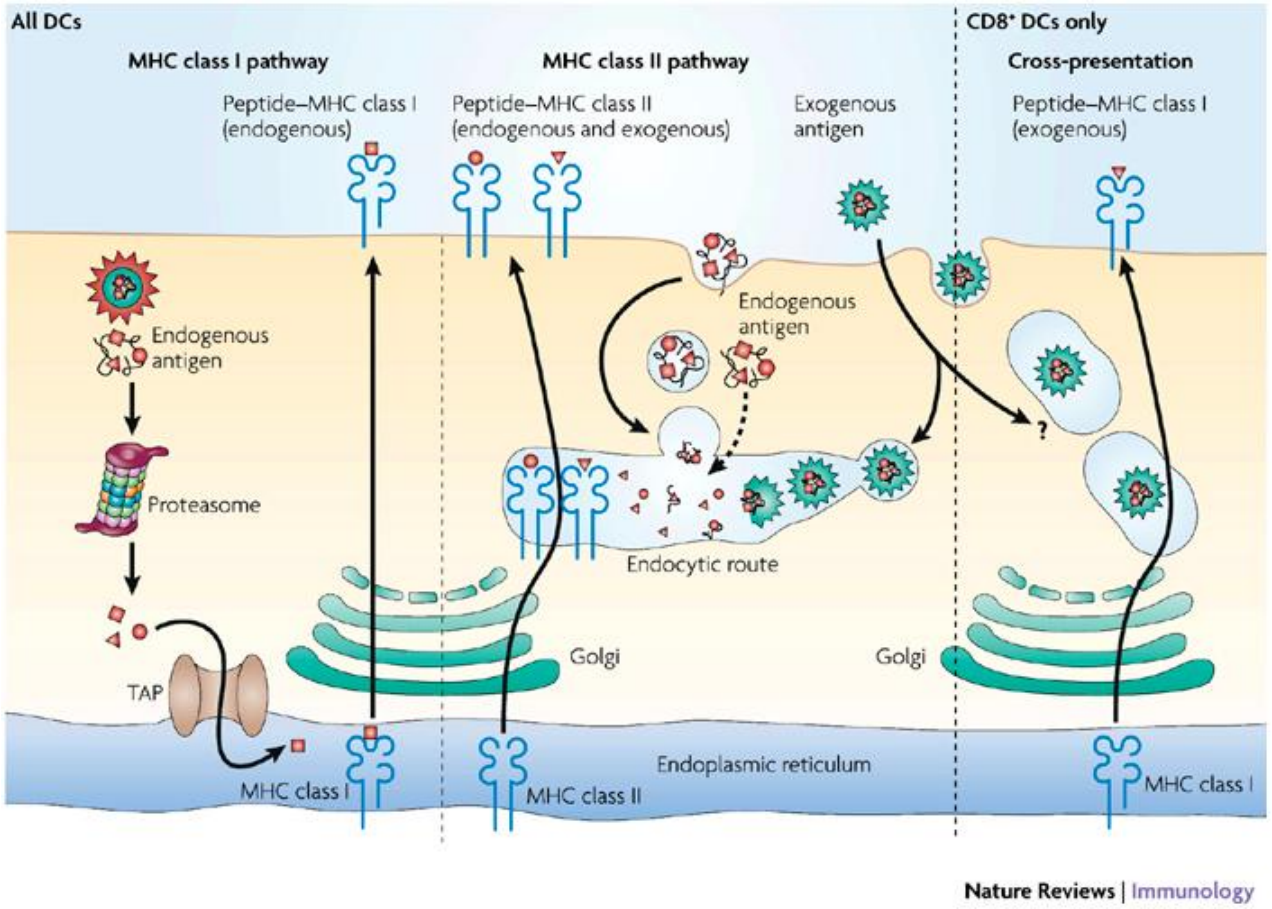
# Yeni aday antijenlerin araştırılması

- Bugüne kadar yeni aşı geliştirme stratejileri, Mtb'e karşı konağın koruyucu bağışık yanıtının arttırılmasına odaklanmıştır.
- Ancak yeni kanıtlar T-hücre temelli koruyucu aşının geliştirilmesinde, kazanılmış immün yanıtın başlatılabilmesi için dendritik hücrelerin de önemli bir role sahip olduğunu göstermiştir.



# Yeni aday antijenlerin araştırılması

- IRGM geni (mmunity-related GTPase family) otofaji geni olarak bilinir ve basil bu şekilde otofaji mekanizmasını bloke edebilir.



# Yeni aday antijenlerin araştırılması

- Yapılan ilk çalışmalarda,

  Mtb'e karşı bağışık yanıtın ölü mikobakteriye karşı değil canlı mikobakteriye karşı oluştuğu gösterilmiştir. Bunun nedeni;

  sadece canlı mikobakteriler tarafından salgılanabilen antijenlerdir.

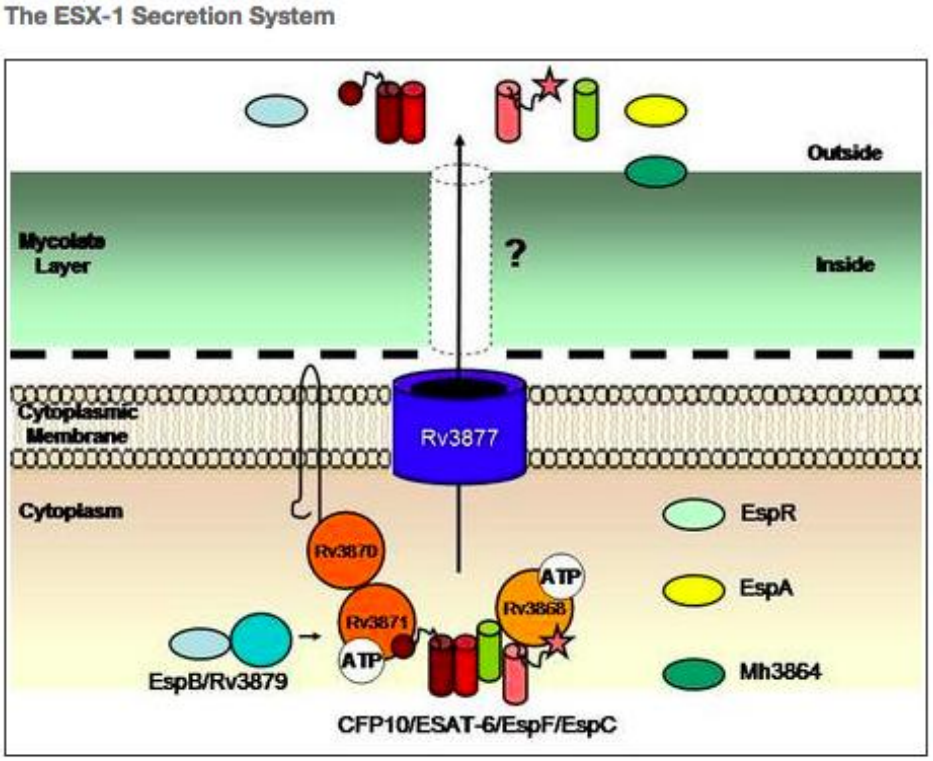



Figure 1: The ESX-1 secretion machine translocates virulence factors across the mycobacterial cytoplasmic membrane. Rv3877 is a multi-transmembrane protein that likely contributes to the formation of a trans-membrane pore. There are three AAA ATPases associated with ESX-1, including Rv3870, Rv3871 and Rv3868, which likely provide energy for secretion. Substrates include the CFP-10/ESAT-6 pair, EspC, EspF, EspA, EspB, EspR and Mh3864. Our work supports a model in which C-terminal regions of ESX-1substrates function to target them to cognate ATPases, either directly or through protein interaction

# *Culture filtrate protein* (CFP)

- *M. tuberculosis'in* birçok farklı antijeni, basil kültürde üredi̇ğinde kültür ortamına salınmaktadır. Filtreden geçebilen bu antijenlere "*culture filtrate protein*" (CFP) adı verilmektedir.

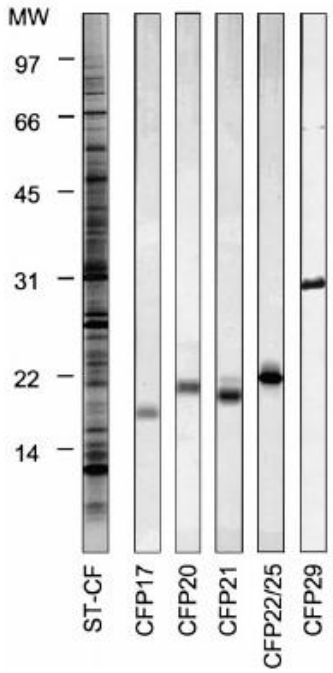


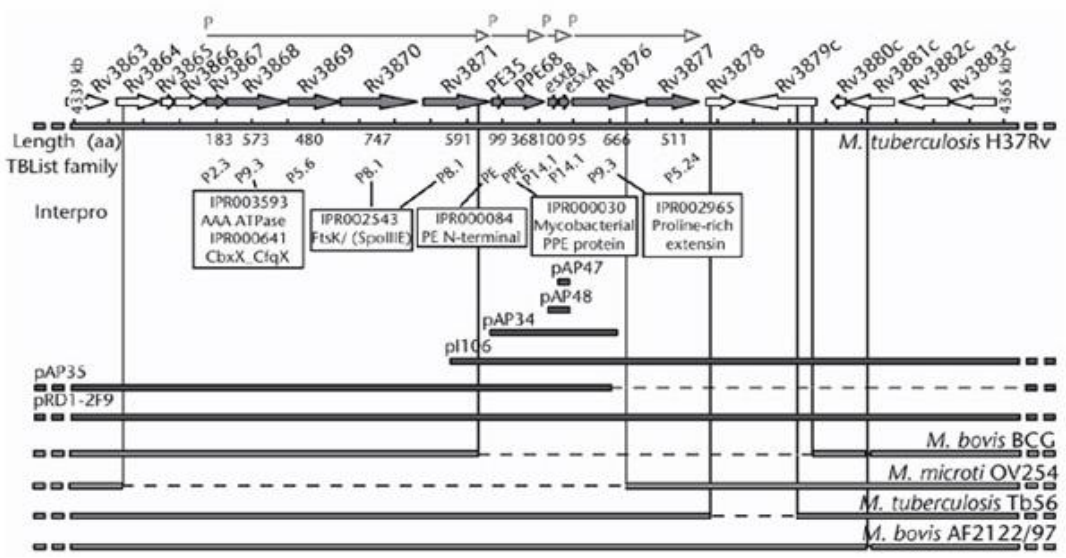

Fig. 2. SDS-PAGE separation of single proteins purified from ST-CF. The preparations were separated in non-reducing SDS-PAGE followed by silver staining. CFP22 and CFP25 were co-purified as one band. Sizes of molecular mass markers (in kilodaltons) are indicated on the left.

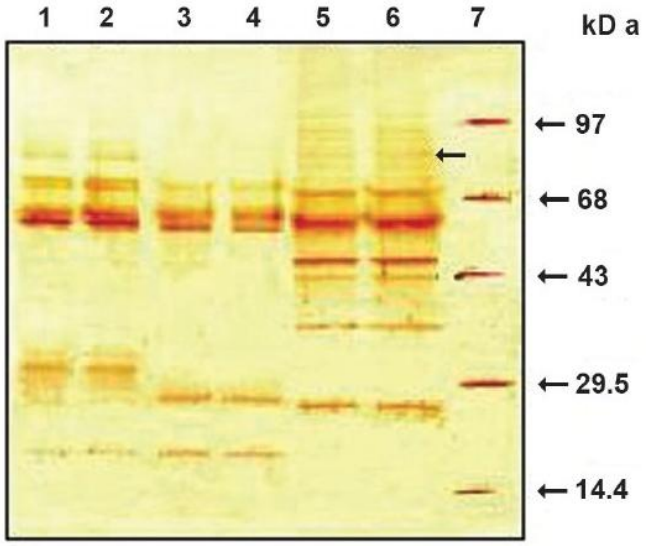
# *Culture filtrate protein* (CFP)

- Bu proteinler immün sistem tarafından ilk tanınan proteinlerdir

  ve lenfosit yanıtı bu proteinlere karşı gelişir.

  Bu proteinler; Mtb genomunun RD1 lokusunda kodlanmıştır.



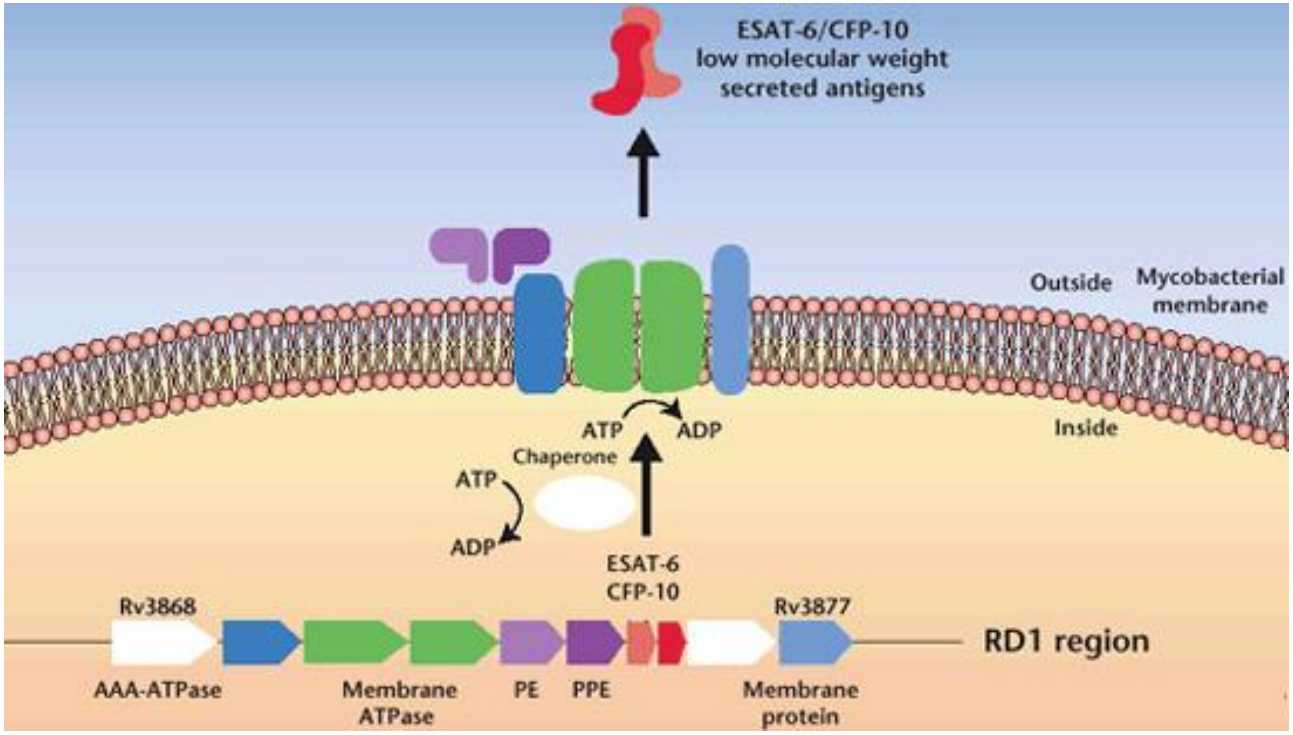

- RD1 lokusu;

  BCG suşunda ve tüberküloz dışı mikobakterilerde bulunmamaktadır. Bu yüzden bu proteinler hem aşı ve hem de tanı testlerinin geliştirilmesi için iyi bir adaydır.

# *Culture filtrate protein* (CFP)

- Yapılan ilk çalışmalarda RD1 lokusunun,

  CFP-10 (10-kDa culture filtrate protein) ve Esat-6 (6-kDa *early secretory antigenic target*) olmak üzere iki salgılanabilir protein kodladığı gösterilmiştir.



# *Culture filtrate protein* (CFP) Ag85

- Kültür filtrat proteinlerinin en önemlilerinden biri de antijen 85 kompleksi (Ag85) üyeleridir.
- Hem Mtb hem de BCG suşunda bulunan bu salgısal proteinler; Ag85A (32 kDa), Ag85B (30 kDa) ve Ag85C (32.5 kDa) olmak üzere genomun üç farklı bölgesinde kodlanmıştır.

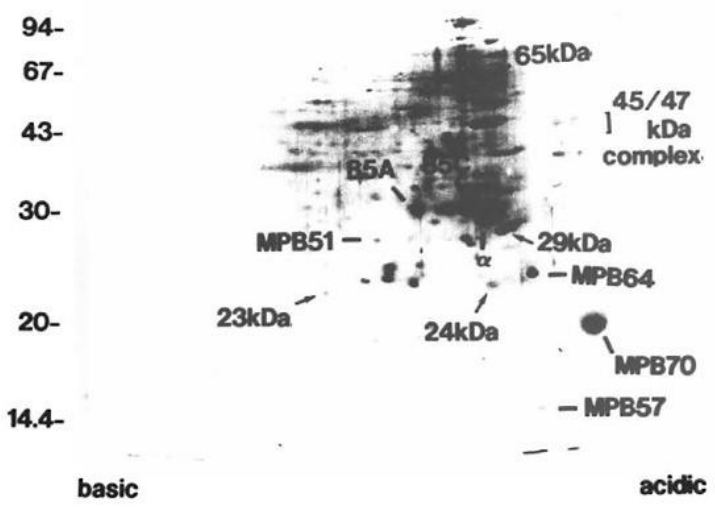



Fig. 1. 2-DE of CF. proteins derived from BCG. The gel was visualized by silver staining. Apparent sizes ofmolecular weight standards are indicated in kDa along the left side. 85A = Ag85-A; α = antigen; 85C = Ag85-C; 65 kDa = 65-kDa protein (groEL analog); MPB = major protein secreted from BCG.

# *Culture filtrate protein* (CFP) Ag85

- Bunlardan Ag85A ve Ag85B başlıca salgısal Mtb proteinleridir ve hayvan modellerinde koruyucu immün yanıtta anahtar rol oynadıkları gösterilmiştir.

*Proc. Natl. Acad. Sci. USA*
Vol. 92, pp. 1530–1534, February 1995
Microbiology

## Protective immunity against tuberculosis induced by vaccination with major extracellular proteins of *Mycobacterium tuberculosis*

MARCUS A. HORWITZ*, BYONG-WHA ESTHER LEE, BARBARA JANE DILLON, AND GÜNTER HARTH

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*Communicated by Emil C. Gotschlich, The Rockefeller University, New York, NY, October 20, 1994*

**ABSTRACT** **Tuberculosis, caused by the intracellular pathogen *Mycobacterium tuberculosis*, is the world's leading cause of death in humans from a single infectious agent. A safe and effective vaccine against this scourge is urgently needed. This study demonstrates that immunization with the 30-kDa major secretory protein, alone or in combination with other abundant extracellular proteins of *M. tuberculosis*, induces strong cell-mediated immune responses and substantial protective immunity against aerosol challenge with virulent *M. tuberculosis* bacilli in the highly susceptible guinea pig model of pulmonary tuberculosis. Protection is manifested by decreased clinical illness including decreased weight loss, reduced mortality, and decreased growth of *M. tuberculosis* in the lungs and spleens of immunized animals compared with sham-immunized controls. This study demonstrates that purified major extracellular proteins of *M. tuberculosis* are candidate components of a subunit vaccine against tuberculosis and provides compelling support for the concept that extracellular proteins of intracellular pathogens are key immunoprotective molecules.**

The design of a subunit vaccine against *M. tuberculosis* must take into account certain features of this pathogen that distinguish it from most others against which successful vaccines have been developed. First, and most importantly, *M. tuberculosis* is an intracellular pathogen that survives and multiples within mononuclear phagocytes. It is phagocytized via complement and mannose receptors on the host cell surface (4, 5). Thereafter, the organism resides intracellularly in a membrane-bound phagosome that has class II major histocompatibility complex (MHC) molecules and endosomal characteristics, including transferrin receptors (6), but does not fuse with lysosomes (6, 7). Second, cell-mediated immunity, as opposed to humoral immunity, plays a dominant role in host defense against *M. tuberculosis* and other intracellular pathogens.

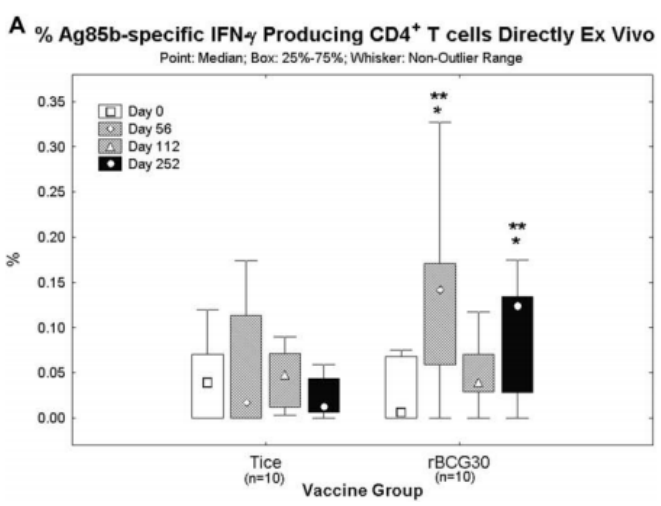
Intracellular pathogens, including *M. tuberculosis* and the intracellular bacterial parasite *Legionella pneumophila*, secrete or otherwise release proteins into their phagosomes within human mononuclear phagocytes (refs. 8 and 9; D. L. Clemens and M.A.H., unpublished data). These facultative intracellular pathogens also release these proteins, collectively referred to

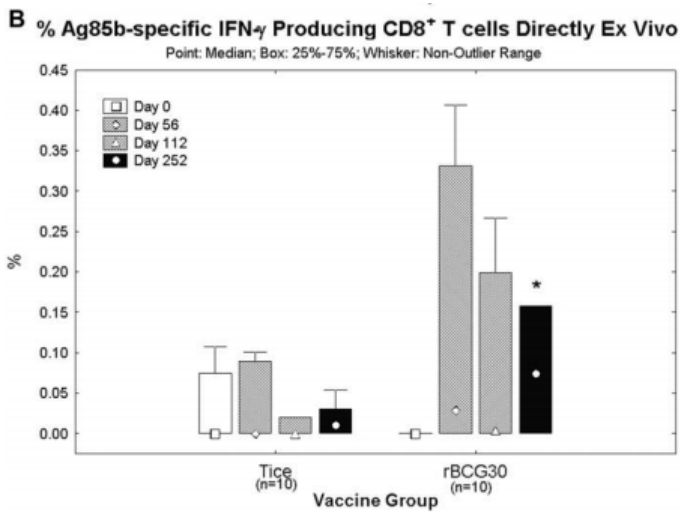
# *Culture filtrate protein* (CFP) Ag85

- C57BL/6 farelerinde Ag85B epitopuna karşı T-lenfositlerinin yüksek düzeyde IFN-γ ve IL-2 ürettiği gösterilmiştir.

## A New Recombinant Bacille Calmette-Guérin Vaccine Safely Induces Significantly Enhanced Tuberculosis-Specific Immunity in Human Volunteers

Daniel F. Hoft,[1] Azra Blazevic,[1] Getahun Abate,[1] Willem A. Hanekom,[5] Gilla Kaplan,[2] Jorge H. Soler,[2] Frank Weichold,[3] Larry Geiter,[3] Jerald C. Sadoff,[3] and Marcus A. Horwitz[4]



# *Culture filtrate protein* (CFP) Ag85

- Viral vektörler ya da protein adjuvanlar ile geliştirilen Ag85 içeren aşılar, klinik çalışmalarda BCG aşısı yerine kullanılmaktadır.
- Bu çalışmalardan biri, Ag85 eksprese eden, rekombinant modifiye aşı virüsü Ankara (*modified vaccinia virus* Ankara 85A, MVA85A) ile gerçekleştirilmektedir.

INFECTION AND IMMUNITY, Feb. 2001, p. 681–686 Vol. 69, No. 2
0019-9567/01/$04.00+0 DOI: 10.1128/IAI.69.2.681–686.2001


## Enhanced Immunogenicity of CD4$^+$ T-Cell Responses and Protective Efficacy of a DNA-Modified Vaccinia Virus Ankara Prime-Boost Vaccination Regimen for Murine Tuberculosis

HELEN McSHANE,[1,2]* ROGER BROOKES,[1] SARAH C. GILBERT,[2] AND ADRIAN V. S. HILL[1,2]

*Nuffield Department of Medicine, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DU,[1] and Wellcome Trust Centre for Human Genetics, University of Oxford, Roosevelt Drive, Oxford OX3 7BN,[2] United Kingdom*

Received 21 July 2000/Returned for modification 25 September 2000/Accepted 28 October 2000

# *Culture filtrate protein* (CFP) Ag85A

- Faz IIb aşamasında olan çalışma sonucunda, geliştirilen MVA85A aşısının BCG ile aşılanmış sağlıklı çocuklarda etkili ve güvenli olduğu gösterilmiştir.

## Safety and efficacy of MVA85A, a new tuberculosis vaccine, in infants previously vaccinated with BCG: a randomised, placebo-controlled phase 2b trial

*Michele D Tameris*, Mark Hatherill*, Bernard S Landry, Thomas J Scriba, Margaret Ann Snowden, Stephen Lockhart, Jacqueline E Shea, J Bruce McClain, Gregory D Hussey, Willem A Hanekom, Hassan Mahomed†, Helen McShane†, and the MVA85A 020 Trial Study Team*

**Summary**

**Background** BCG vaccination provides incomplete protection against tuberculosis in infants. A new vaccine, modified Vaccinia Ankara virus expressing antigen 85A (MVA85A), was designed to enhance the protective efficacy of BCG. We aimed to assess safety, immunogenicity, and efficacy of MVA85A against tuberculosis and *Mycobacterium tuberculosis* infection in infants.

*Lancet* 2013; 381: 1021–28
Published Online
February 4, 2013
http://dx.doi.org/10.1016/S0140-6736(13)60177-4

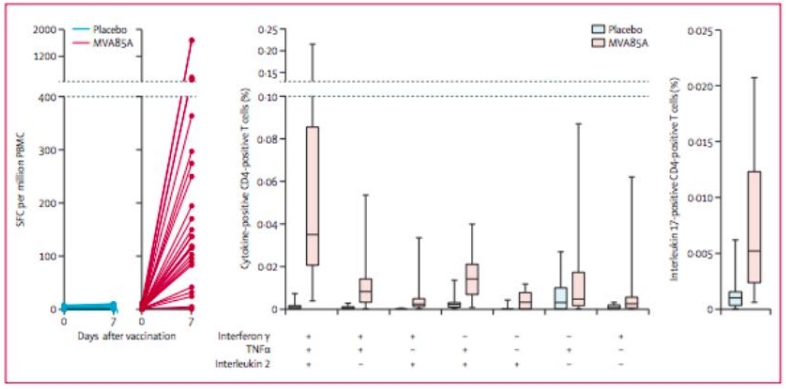



**Figure 2: Vaccine immunogenicity**
(A) Frequencies of Ag85A-specific T cells measured by interferon-γ enzyme-linked immunosorbent spot assay in infants in study group 2 (27 infants in the MVA85A group and 27 infants in the placebo group) before administration of placebo or MVA85A (day 0) and 7 days after vaccination. (B) Frequencies of cytokine-expressing Ag85A-specific Th1 (CD4-positive T cells expressing IFN-γ, TNFα, or interleukin 2) and (C) frequencies of Ag85A-specific Th17 (CD4-positive T cells expressing interleukin 17) cells, measured by whole blood intracellular cytokine staining 28 days after administration of placebo or MVA85A to infants in study group four (17 infants in the MVA85A group and 19 infants in the placebo group). SFC=spot-forming cells. PBMC=peripheral blood mononuclear cell.

# *Culture filtrate protein* (CFP) Ag85B

- Yapılan diğer bir ilginç çalışmada ise,

  Ag85B ve iki Mtb plazmidi DNA aşısı olarak kullanılmıştır. Bu DNA aşısı ile hücrede otofaji indüklenmiş sunulması sonucunda yüksek antikor cevabı ve bununla birlikte dalakta IFN-γ ve IL-2 üretimi tespit edilmiştir.

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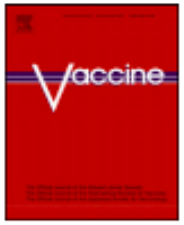



## Enhancement of immune response to a DNA vaccine against *Mycobacterium tuberculosis* Ag85B by incorporation of an autophagy inducing system

Q1 Jomkhwan Meerak[a,b], Supason Wanichweacharungruang[c], Tanapat Palaga[a,*]

[a] *Department of Microbiology, Faculty of Science, Chulalongkorn University, Phayathai Road, Bangkok 10330, Thailand*
[b] *Division of Microbiology, Department of Biology, Faculty of Science, Chiang Mai University, Huaykaew Road, Chiangmai 50200, Thailand*
[c] *Department of Chemistry, Faculty of Science, Chulalongkorn University, Phayathai Road, Bangkok 10330, Thailand*

# *Culture filtrate protein* (CFP) ESAT-6

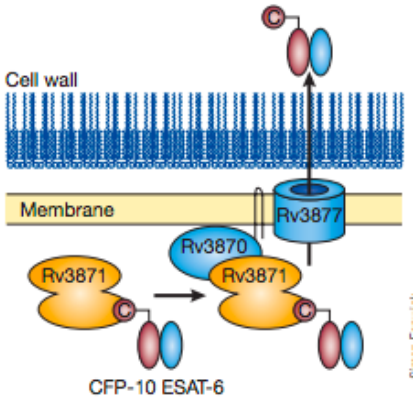
- Diğer bir önemli CFP proteini de yine RD1 lokusunda kodlanan,
- düşük moleküler ağırlıklı (6 kD) ESAT-6 (*early secreted antigenic target*-6) salgısal proteinidir.

NEWS AND VIEWS

## Mycobacterial virulence and specialized secretion: same story, different ending

Marcel A Behr & David R Sherman

**The genomic region lost during the attenuation of BCG vaccine encodes a newly discovered secretion system conserved among gram-positive bacteria. A series of papers has now dissected the components of this system, revealing a unique *Mycobacterium tuberculosis*–specific signal for export of bacterial proteins into the host.**



# *Culture filtrate protein* (CFP) ESAT-6

- Yapılan deneysel çalışmalarda;

  hem CD4+ hem de CD8+ T lenfositlerini indükleyebildiği ve BCG ile yapılan immünizasyon kadar etkin bağışıklık sağladığı gösterilmiştir.

## Recombinant BCG exporting ESAT-6 confers enhanced protection against tuberculosis

Alexander S. Pym[1,2], Priscille Brodin[1], Laleh Majlessi[3], Roland Brosch[1], Caroline Demangel[1], Ann Williams[4], Karen E. Griffiths[4], Gilles Marchal[5], Claude Leclerc[3] & Stewart T. Cole[1]

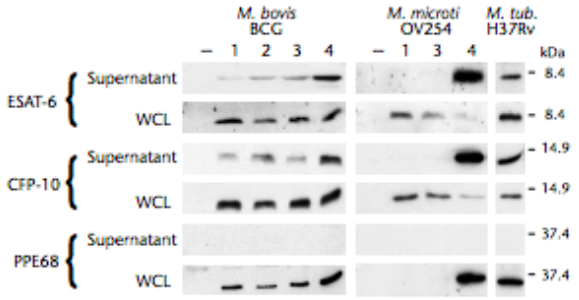



**Fig. 2** Western blot analysis of various RD1 knock-ins of *M. bovis* BCG and *M. microti*. Left, results of immunodetection of ESAT-6, CFP-10 and PPE68 (Rv3873) in whole-cell lysates (WCL) and culture supernatants of BCG recombinants; center, equivalent findings from *M. microti*; right, *M. tuberculosis* (*M. tub.* H37Rv) control samples. Samples were obtained from mycobacteria, transformed with various plasmids, grown in parallel. Equal quantities of protein were present in each of the lanes. Lane numbers correspond to the following: –, pYUB412 vector control; 1, pAP34; 2, pAP35; 3, RD1-I106; 4, RD1-2F9. The positions of the nearest molecular weight markers are indicated.

# *Culture filtrate protein* (CFP) ESAT-6 Ag85B

- Faz I klinik çalışmaları devam eden

  Hibrit I aşısı hem Ag85B hem de ESAT-6 içermektedir.

  Hayvan modelleri ile yapılan çalışmalarda iki farklı güçlü antijenik komponent taşıyan bu aşının Mtb'e karşı güçlü koruyucu etkisinin olduğu gösterilmiştir.

- Çalışmalarda tüberküloza karşı geliştirilen Ag85b ve ESAT-6 içeren subünit aşılarının, bellek CD4+ T lenfositlerinin proliferasyonunu sağladığı gösterilmiştir.

# *Culture filtrate protein* (CFP) TB10.4

- Kültür filtrat proteinlerin araştırılmasında keşfedilen diğer bir antijen TB10.4 (Rv0288) proteinidir.
- Tüberküloz hastalarında ve BCG ile aşılanmış kişilerde bu proteine karşı ESAT-6'da olduğu gibi güçlü tanıma gerçekleşmektedir.

# *rekombinant urease-deficient BCG (BCG-dHCM)*

- heat shock protein (HSP)70,
- MTB-derived CysO ve
- major membrane protein (MMP)-II üreten rekombinant BCG suşu

IFN-γ yanıtı, dendritik hücrelerde aktivasyonu,

antijen spesifik olarak CD4+ ve CD8+ T hücrelerinde farklılaşma

# *rekombinant urease-deficient BCG (BCG-dHCM)*

- Araştırmacılar çalışmalarının sonucunda;

  HSP70, CysO, MMp-II antijenlerinin

  poliklonal T hücre aktivasyonunu sağladığını belirtmişlerdir.

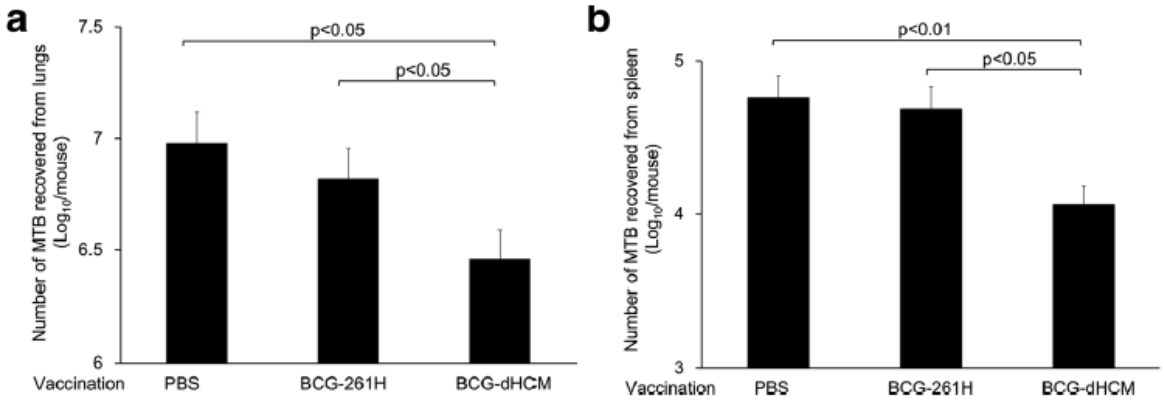



**Figure 7 Inhibition of MTB multiplication by subcutaneous vaccination with BCG-dHCM.** Five-week-old C57BL/6 mice (5 mice/group) were subcutaneously vaccinated with $1 \times 10^3$ CFU/mouse of either BCG-261H or BCG-dHCM and were challenged with 100 CFU/lungs of H37Rv by aerosol infection, at 6 weeks post vaccination. The number of MTB recovered from the lung **(a)** or spleen **(b)** at 6 weeks post challenge was enumerated by colony assay method. Representative results of three separate experiments are shown. Titers were statistically compared using Student's *t*-test.

# *InsB proteini*

- Park ve arkadaşları yaptıkları çalışmada, aşı adayı antijenleri araştırmak amacıyla Mtb K suşu, H37Rv suşu ve non-Beijing izolatlarının genomik yapılarını karşılaştırmışlardır.

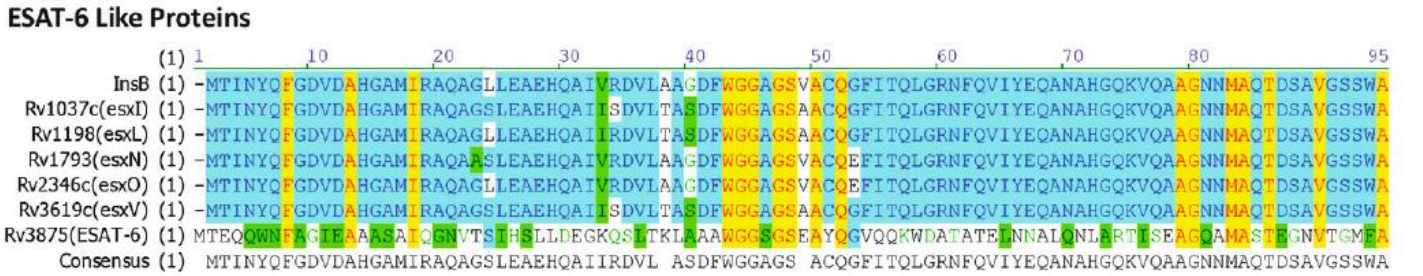



**Fig. 2. Alignment of InsB compared with related proteins in the family and ESAT-6 of *M. tuberculosis* H37Rv.** Sequence analysis of the K-specific InsB protein shows differences only in several amino acids when compared to other related ESAT-6-like proteins, except the ESAT-6 sequence found in the H37Rv genome (Rv3875). The alignment result of amino acid sequences between ESAT-6 and InsB showed only consensus amino acid sequence conservation in each antigen.

# *InsB proteini*

- Oluşturulan hayvan modelinde, klonlanarak *Escherichia coli*'ye aktarılan ve eksprese edilen InsB proteinin, güçlü bir IFN-γ ve IL-17 cevabı oluşturduğu ancak buna karşın IL-2 yanıtının zayıf olduğu gösterilmiştir.

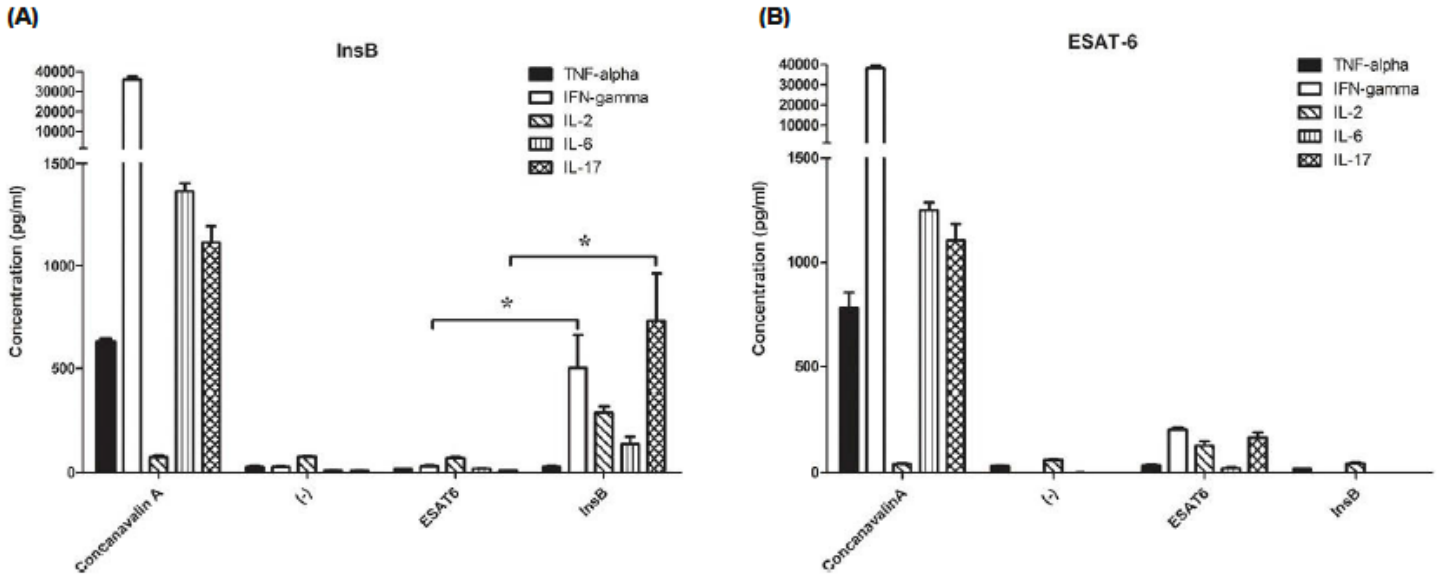



**Fig. 5. Cytokine analysis of mice immunized with InsB or ESAT-6 protein.** Splenocytes from mice immunized with InsB (A) or ESAT-6 (B) were stimulated with either medium (RPMI with 10% FBS), InsB, or ESAT-6, and their supernatants were analyzed by flow cytometry.

# *heparin-binding hemagglutinin (HBHA)*

- Schepers ve arkadaşları yaptıkları çalışmada,

  bir aşı adayı olarak mikobakteriyel heparin-binding hemagglutinin (HBHA) antijenini erken hücresel immün cevaptaki etkinliğini tüberkülozlu çocuklarda araştırmışlardır.

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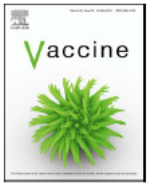


## Early cellular immune response to a new candidate mycobacterial vaccine antigen in childhood tuberculosis

K. Schepers[a,b], V. Dirix[a], F. Mouchet[c], V. Verscheure[a], S. Lecher[d,e,f,g], C. Locht[d,e,f,g], F. Mascart[a,h,*]

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[b] Immunodeficiency Unit, Hôpital Erasme, Université libre de Bruxelles (U.L.B.), Brussels, Belgium
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[h] Immunobiology Clinic, Hôpital Erasme, Université Libre de Bruxelles (U.L.B.), Brussels, Belgium

# *heparin-binding hemagglutinin (HBHA)*

- Bu çalışmada, hem saflaştırılan doğal HBHA hem de rekombinant HBHA ile tüberkülozlu çocuklarda INF-? yanıtının geliştiği ve BCG aşısının etkinliğinin arttığı bildirilmektedir.

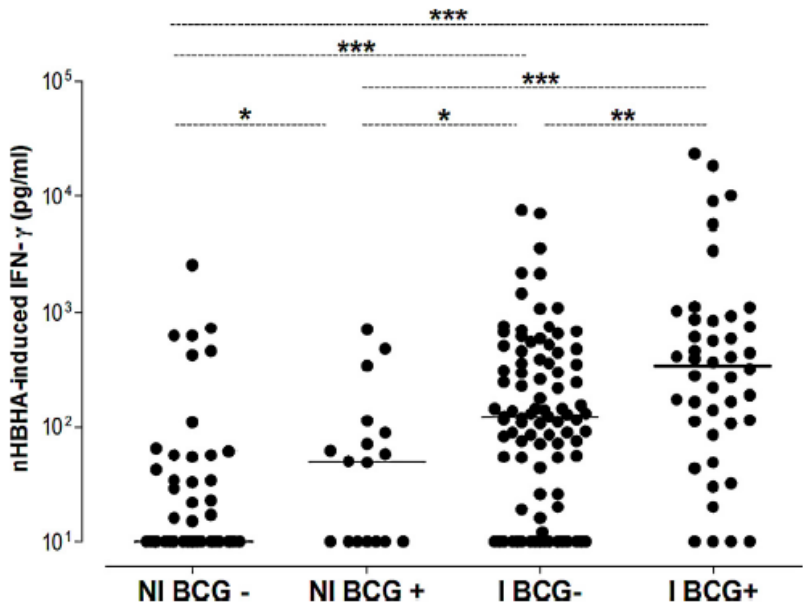



**Fig. 2.** *Higher nHBHA-induced IFN-γ concentrations in BCG-vaccinated infected children compared to non BCG-vaccinated infected or to BCG-vaccinated non-infected children.* PBMC were isolated from NI controls ($n = 89$, left) and from *Mtb*-infected (I) children (aTB and LTBI, $n = 134$, right). Children were stratified into 2 groups according to the BCG vaccination status, vaccinated ($n = 17$ NI and $n = 44$ I, BCG+) or not vaccinated ($n = 72$ NI and $n = 90$ I, BCG−). PBMC were *in vitro* stimulated with 2 μg/ml nHBHA and IFN-γ concentrations were measured in 96 h cell culture supernatants. Filled horizontal lines represent the medians, * $p = 0.02$, ** $p = 0.006$, *** $p < 0.001$.

# *MT1721, MT1694, MT2462 ve MT3444 antijenleri*

- Aşı adayı antijenlerin belirlenmesinde yeni bir yaklaşım da,
  tüberkülozlu insan ve hayvanların çeşitli vücut sıvılarında Mtb'a ait antijenlerin aranmasıdır.
- Yüksek performans sıvı kromatografisi (HPLC) ve kütle spektrometrik cihazlarla yapılan araştırmalarda, akciğer tüberkülozlu hastaların akciğer dokularında ve idrar örneklerinde MT1721, MT1694, MT2462 ve MT3444 antijenleri tespit edilmiştir.

# *MT1721, MT1694, MT2462 ve MT3444 antijenleri*

- Bu potansiyel aşı adayı antijenlerin,

  CD4+ ve CD8+ T hücreleri üzerine etkisinin araştırıldığı birçok çalışma halen devam etmektedir.

Immunology
The Journal of cells, molecules, systems and technologies
British Society for immunology
IMMUNOLOGY ORIGINAL ARTICLE

## Robust immune response elicited by a novel and unique *Mycobacterium tuberculosis* protein using an optimized DNA/protein heterologous prime/boost protocol

Mark J. Cayabyab,[1,2,*] Suely S. Kashino[1,*] and Antonio Campos-Neto[1]

[1]*Global Infectious Disease Research Center, The Forsyth Institute, Cambridge, MA, and* [2]*Department of Oral Medicine, Infection and Immunity, Harvard School of Dental Medicine, Boston, MA, USA*

doi:10.1111/j.1365-2567.2011.03525.x
Received 1 July 2011; revised 22 September 2011; accepted 13 October 2011.
*These authors contributed equally to this work.
Correspondence: A. Campos-Neto, Global Infectious Disease Research Center, Forsyth Institute, 245 First Street, Cambridge, MA 02142, USA.

**Summary**

An efficacious tuberculosis (TB) vaccine will probably need to induce both CD4 and CD8 T-cell responses specific to a protective *Mycobacterium tuberculosis* antigen(s). To achieve this broad cellular immune response we tested a heterologous DNA/protein combination vaccine strategy. We used a purified recombinant protein preparation of a unique *M. tuberculosis* antigen (rMT1721) found in the urine of TB patients, an optimized plasmid DNA expressing this protein (DNA-MT1721), and a Toll-like receptor 4 agonist adjuvant. We found that priming mice with DNA-MT1721 and subsequently boosting with rMT1721 elicited high titres of specific IgG1 and IgG2a antibodies as well as high magnitude and polyfunctional $CD4^+$ T-cell responses. However, no detectable $CD8^+$ T-cell response was observed using this regimen of immunization. In contrast, both $CD4^+$ and $CD8^+$ T-cell responses were detected after a prime/boost vaccination regimen using rMT1721 as the priming antigen and DNA-MT1721 as the boosting immunogen. These findings support the exploration of heterologous DNA/protein immunization strategies in vaccine development against TB and possibly other infectious diseases.

# *MT1721, MT1694, MT2462 ve MT3444 antijenleri*

- Cayabyab ve arkadaşlarının MT1721 antijenini eksprese eden DNA aşısıyla yaptıkları çalışmada, bu antijenin hem CD4+ Th1 hem de CD8+ T hücrelerinde etkili bir immün yanıt oluşturduğu gösterilmiştir.

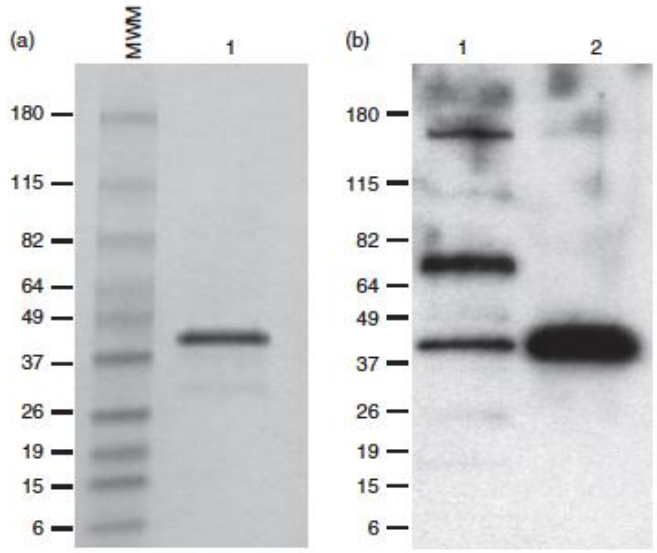
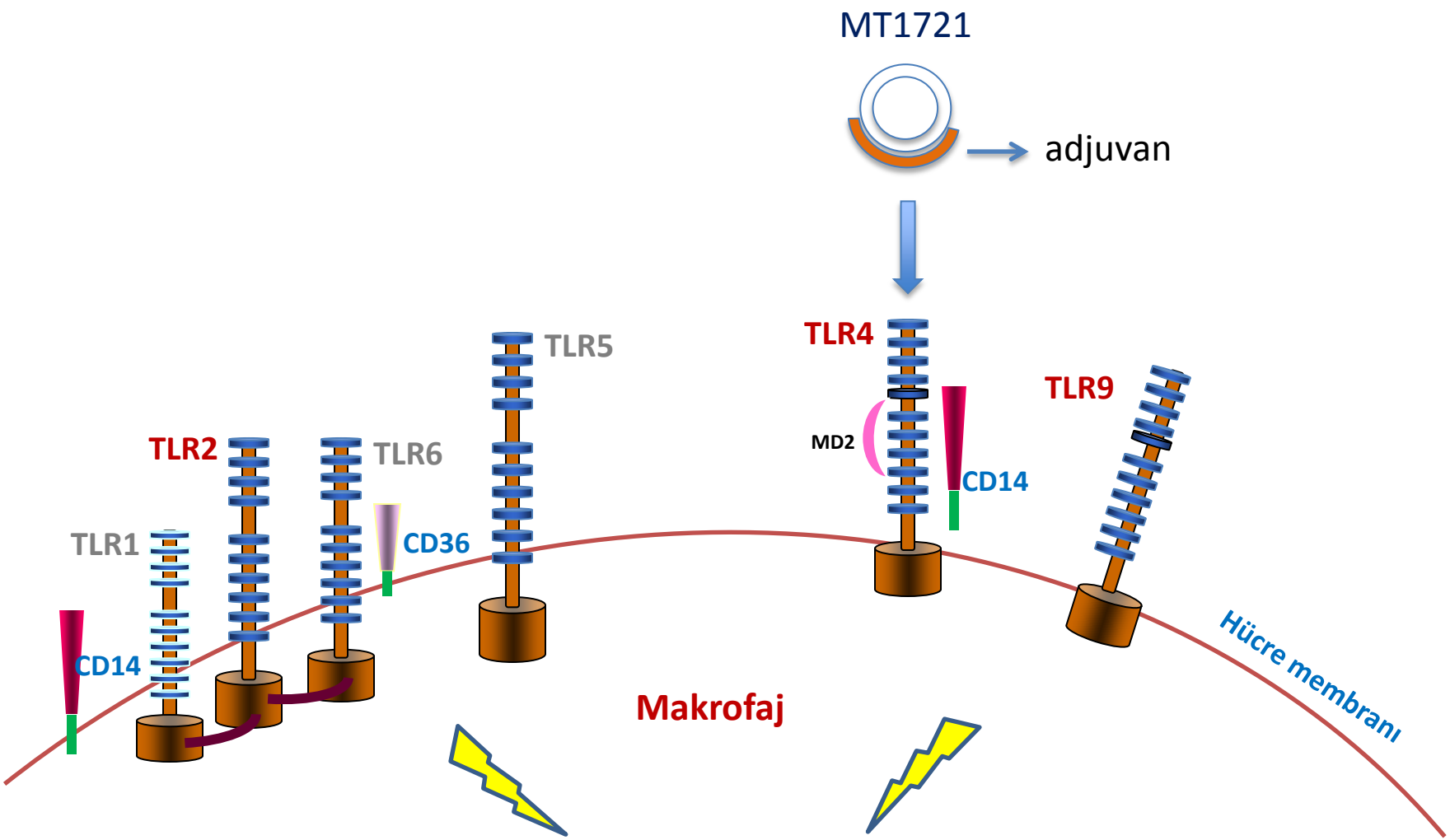



**Figure 1.** Identification of native MT1721 antigen in the culture filtrate of *Mycobacterium tuberculosis*. Purified rMT1721 was electrophoresed under reducing conditions in 4–20% gradient SDS–PAGE and stained with Coomassie Blue (a). In parallel, rMT1721 and culture filtrate samples were run in SDS–PAGE under similar conditions and transferred to a PVDF membrane followed by probing with rabbit anti-rMT1721 antiserum. Reactivity was detected with horseradish peroxidase-labelled goat anti-rabbit IgG and developed using the enhanced chemiluminescent reagent (b). Lane 1, culture filtrate; lane 2, purified rMT1721.

# *MT1721, MT1694, MT2462 ve MT3444 antijenleri*



# *MT1721, MT1694, MT2462 ve MT3444 antijenleri*

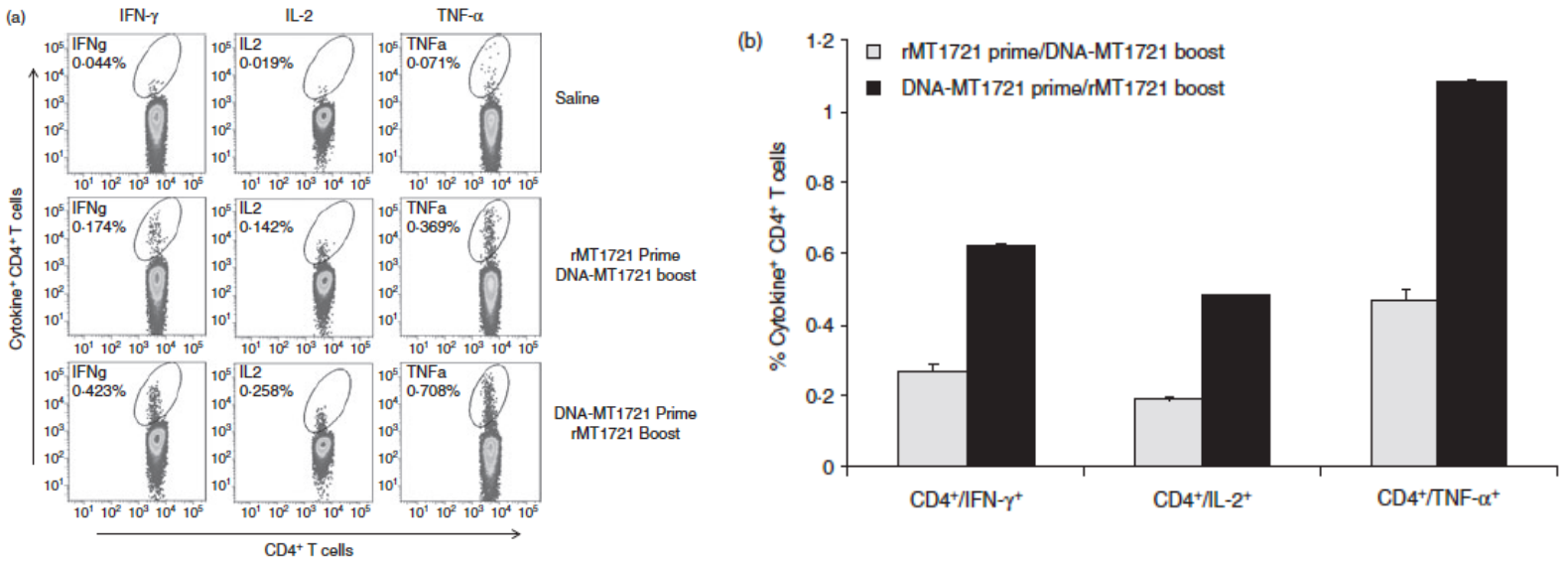



**Figure 4.** Heterologous DNA and recombinant protein prime/boost immunization induces robust *Mycobacterium tuberculosis* rMT1721-specific $CD4^+$ cells producing interferon-$\gamma$ (IFN-$\gamma$), tumour necrosis factor-$\alpha$ (TNF-$\alpha$) and interleukin-2 (IL-2). Splenocytes from three mice per group were isolated 12 days after immunization. The splenocytes were exposed to rMT1721 and cytokine production was measured by monoclonal antibody staining and flow cytometric analysis. (a) Flow cytometer plot of results obtained from one representative individual mouse from each group. (b), Average (± SEM) of the percentage of IFN-$\gamma$-producing, TNF-$\alpha$-producing and IL-2-producing $CD4^+$ T cells obtained from three mice per group following stimulation with rMT1721. Mice receiving the DNA-MT1721 prime/rMT1721 boost generated markedly higher CD4 T-cell responses for all three cytokines (IFN-$\gamma$, $P < 0.021$; TNF-$\alpha$, $P < 0.015$; and IL-2, $P < 0.026$) compared with rMT1721 prime/DNA-MT1721 boost.

# *MTB Genomu ve Biyoinformatik*

- Gomez ve arkadaşları;

  aşı için hedef antijenlerin araştırmak için Mtb genomunu farklı bilgisayar programları ile analiz etmişlerdir.

## Identification of Secreted Proteins of *Mycobacterium tuberculosis* by a Bioinformatic Approach

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**Proteins secreted by *Mycobacterium tuberculosis* are usually targets of immune responses in the infected host. Here we describe a search for secreted proteins that combined the use of bioinformatics and *phoA′* fusion technology. The 3,924 proteins deduced from the *M. tuberculosis* genome were analyzed with several computer programs. We identified 52 proteins carrying an $NH_2$-terminal secretory signal peptide but lacking additional membrane-anchoring moieties. Of these 52 proteins—the TM1 subgroup—only 7 had been previously reported to be secreted proteins. Our predictions were confirmed in 9 of 10 TM1 genes that were fused to *Escherichia coli phoA′*, a marker of subcellular localization. These findings demonstrate that the systematic computer search described in this work identified secreted proteins of *M. tuberculosis* with high efficiency and 90% accuracy.**

# *MTB Genomu ve Biyoinformatik*

- Bu çalışmada,
- Mtb genomunda 3924 adet protein kodlayan gen bölgesi tespit edilmiştir.
- Bunlardan 52'sinin salgısal protein olduğunu ve NH2-terminal sinyal peptidi taşıdığını belirlemişlerdir.

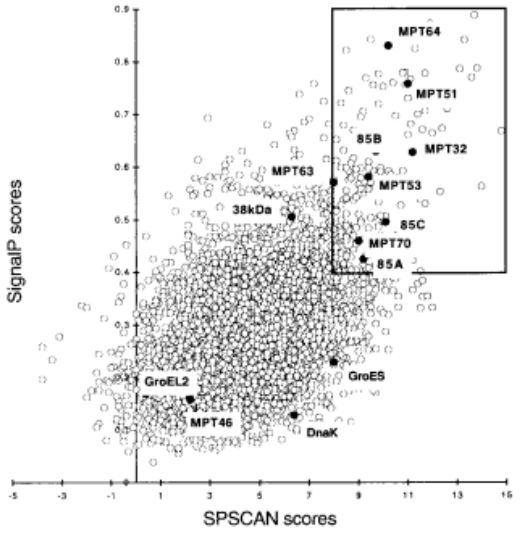


FIG. 3. Correlation between SignalP and SPScan scores. Each circle represents one of the 3,924 deduced proteins of *M. tuberculosis*. The position of the circle indicates the scores assigned by SignalP and SPScan to the signal peptide. For this study, the two computer programs were set in the mode for gram-positive bacteria. SPScan was used in the adjusted mode, which penalizes signal peptides longer than 45 amino acid residues (i.e., the maximal length for gram-positive bacteria). The boxed area contains the Top208 group of proteins that scored ≥0.4 with SignalP and ≥8 with SPScan. The solid circles within the boxed area represent nine known secreted proteins (Ag85A, Ag85B, Ag85C, MPT32, MPT51, MPT53, MPT63, MPT64, and MPB70) (34) that were used to set cutoff values for SignalP and SPScan scores. The solid circles outside of the boxed area represent four known proteins (MPT46, GroES, GroEL2, and DnaK) containing no signal peptide (34). Also outside of the boxed area is located the 38-kDa antigen, a secreted protein which exhibits an LPP type of signal peptide (14).

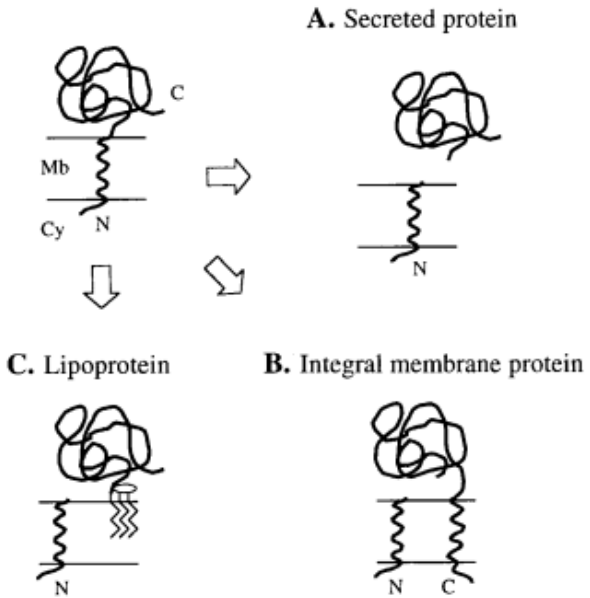


FIG. 1. Fates of proteins having an $NH_2$-terminal signal peptide. The $NH_2$-terminal signal peptide directs translocation of a protein across the cell membrane (top left panel). Processing of the preprotein by a type I signal peptidase releases the mature protein (A), providing that there are no additional transmembrane domains (B). Translocation of LPPs across the membrane is followed by modification with glycerol and fatty acids of the prolipoprotein and processing by a type II signal peptidase. The lipid moiety anchors the LPP to the membrane (C). Mb, cell membrane; Cy, cytoplasm; N, $NH_2$-terminal end; C, COOH-terminal end.

# *Sonuç*

- Yaklaşık 100 yıldır kullanılan ve milyarlarca kişiye uygulanan

  BCG aşısının koruyuculuğu ve etkinliği halen tartışmalıdır.

- Bu tartışmalar altında,

  tüberküloza karşı etkili olabilecek yeni aşı araştırmaları yeni antijen için araştırmalar hem *in-silico* olarak hem de deneysel hayvan modellerinde devam etmektedir.