Experimental Skin Sarcoidosis In A Doctor Volunteer
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Abstract
Scientists couldn't discover the causative agent of sarcoidosis for more than a hundred years. A biopsy of lung taken from a patient with sarcoidosis [Lofgren syndrome] was used to isolate the erythrocyte-like microorganisms [ELM] on human blood agar. It took 5 months of incubation at 37°C and a doctor volunteer producing an experimental sarcoidosis in his skin. The auto-infection resulted in typical sarcoid granulomas and a positive Kveim test 2 years later. It took two auto-infections in 7 days intervals with a suspension of the new microorganism containing 1 million CFU. The sarcoidosis granulomas in the skin biopsy specimens were confirmed by four professors in medicine and the biopsies as well as the ELM cultures were at disposal of every specialist interested in PCR in sarcoidosis and in Crohn's diseases.

INTRODUCTION
Since about one hundred years, the literature is full with articles about sarcoidosis [1]. However, no one has seen experimental sarcoidosis.

Our hypothesis is:

1. Experiments on animals are useless since sarcoidosis is a human disease and this means that auto-infection experiments in human volunteers must be used in order to produce experimental sarcoidosis.

2. The causative agent of sarcoidosis can be seen in sarcoidosis granulomas as the causative agent of tuberculosis can be seen in the tuberculosis granulomas. The problem is that the causative agent of sarcoidosis is quite different in appearance and properties compared to Mycobacterium tuberculosis and all microorganisms known in the literature.

3. The unknown microorganism cannot multiply in the well known nutrient media.

MATERIAL AND METHODS
In May 1993, a transbronchial lung biopsy was taken from a 31-year-old woman with sarcoidosis [Lofgren's syndrome]. The specimen was crushed in 1 ml sterile distilled water and the author inoculated himself with 0.5 ml of this suspension in his left forearm. The remaining 0.5 ml of the suspension was inoculated in tubes with human blood agar, Sabouraud agar, and brain-hearth infusion and incubate at 35°C for 5 months.

RESULTS
The auto-inoculation experiment with a suspension of lung tissue specimen was negative.

K. sarcoidosis was seen for the first time on October 23, 1993 after five months of incubation at 35°C on a Gram stained slide prepared from human blood agar, but not on Sabouraud agar and brain-heart infusion.

Viewed by means of an optical microscope the causative agent of sarcoidosis had a yeast-like appearance. There were several yeast-like microorganisms in a small group in the nutrient medium [Fig 1] exactly as there were several yeast-like Hamazaki-Wesenberg bodies in the sarcoidosis granulomas [Fig. 2]
Electron microscope photographs demonstrate that the causative agent of sarcoidosis has different morphology than all microorganisms known today. This is because the causative agent of sarcoidosis has no visible nucleus or cell walls. Sometimes there are older microorganisms with some white inclusions [Fig. 3] identical to the white inclusions in yellow-brown bodies in sarcoidosis granulomas [Fig. 4]. The younger microorganisms are smaller and have pili which make clear that they are no bodies or erythrocytes [Fig. 4].

The author inoculated approximately 1,000,000 CFU of K. sarcoidosis subcutaneously in the front part of the right leg. After the inoculation, the skin remained normal for a week. This was the reason for a second auto-inoculation with the same doses of the microorganism. However, this times an intradermal way was used. After the second auto infection, there was an immediate reaction and intradermal papula grew and reached 10 mm in diameter with itching and red color as if an insect has bitten the skin. We believe this was an allergic reaction of rapid type.

Several hours later, the skin was normal. Only after 25 days, a violet zone of 8 to 10 cm in diameter was observed on the
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Skin but without any discomfort. There was a red colored place of the skin of 3 cm in diameter in the middle of the violet skin zone, again without any feeling of discomfort. After 35 days, the colored skin showed a tendency to heal and a skin biopsy was performed.

Sarcoid granulomas were found in the histologic examination [Fig.5, 6].

**Figure 5**
Figure 5: Sarcoidosis granulomas in experimental skin sarcoidosis

**Figure 6**
Figure 6: Sarcoidosis granulomas in experimental skin sarcoidosis

In order to recover K. sarcoidosis from the inoculated skin a specimen from the biopsy was inoculated in a brain heart infusion broth with 10% human blood chocolate incubated at 35. After 3 months incubation a Gram stained slid demonstrated spherules of K. sarcoidosis in the chocolate medium.

**K. SARCOIDOSIS CULTURAL PROPERTIES**
K.sarcoidosis was recovered only on human agar. No colonies were seen but a Gram stained slide revealed the new microorganism as yeast-like cells.

Subcultures in brain heart broth, thioglycolate medium, or Sabouraud agar produced no growth unless 5-10% human blood native was added to the medium. Observations with a stereomicroscope revealed that colonies of K.sarcoidosis on human blood agar are smaller than the colonies of M. Pneumoniae. This fact explains why there were no visible colonies on the human blood agar when K.sarcoidosis was isolated for the first time. The microcolonies were gray, soft, plain, or prominent. They could be easily removed with a loop.\[2\,3\]

was washed three times in distilled water and approximately 10,000,000 CFU were suspended in 0.4% formol. Three days later, the suspension was used as Kveim test antigen in an intradermal inoculation in the author's skin.

After two months, a skin biopsy was performed and the sarcoid granulomas were confirmed again[ Fig.7, 8].

**Figure 7**
Figure 7: Sarcoidosis granulomas in Kveim test

**Figure 8**
Figure 8: Sarcoidosis granulomas in Kveim test

Two years after the experimental, a culture of K.sarcoidosis
Identification of the new microorganism is easy. There is no other microorganism in the literature that cannot grow on sheep blood agar and forms fast invisible colonies on human blood agar only. The results can be checked after 20-30 days using a Gram stained slide. There is no growth at room temperature.

Subcultures of K.sarcoidosis in human blood media were examined on electron micrographs in order to study the cycle of development of the new microorganism. It could be seen that K.sarcoidosis are inside a human erythrocytes like in nest [4]. It was also found that K. sarcoidosis uses mimicry. However, outside the erythrocytes the “nucleus” is pushed away and there is a cell without a nucleus like the erythrocyte surrounding the cell [mimicry]. Examining several photographs one can see that K. sarcoidosis has a cycle of development and can penetrate through the erythrocyte's cell wall.

DISCUSSION

An auto-infection experiment with a suspension containing sarcoid granulomas from a transbronchial lung biopsy specimen failed. Small numbers of the causative agent of sarcoidosis inside the granulomas and low virulence of this microorganisms are the possible explanations. A second experiment with 1,000,000 CFU of a pure culture of K. sarcoidosis inoculated twice was a success and produced sarcoid granulomas.

The experiment revealed that human blood is the clue of the one hundred year-old mystery about the causative agent of sarcoidosis. Native human blood in an agar medium isolated K. sarcoidosis from the lung biopsy specimen while the auto-infection experiment failed because the granulomas contained only a small number of K. sarcoidosis.

We believe that sarcoidosis and Crohn's disease both have ELM as causative agents. However, a PCR is needed to proof this hypothesis.

K. sarcoidosis fulfills the three rules of Henle-Koch because it was isolated in pure culture from a piece of sarcoid granulomas, an experimental sarcoidosis was performed with the pure culture, and the same microorganism was isolated from the experimental sarcoidosis produced in a human volunteer.

K. sarcoidosis is the real Kveim test antigen and a formol suspension produced typical sarcoid granulomas in author's skin.

There are many unsolved problems regarding the new group of ELM microorganisms:

- First, DNA analyses are needed to legalize K. sarcoidosis as a new microorganism but only scientist with great skills can perform these DNA analyses. Some preliminary investigations indicate that ELM have a special DNA resembling in some physical properties to the DNA of eucaryotes.
- PCR is extremely important in order to confirm that sarcoidosis and Crohn's disease both have ELM as causative agents.
- K. sarcoidosis is an ELM and has the morphology of an ELM. However, there are other types of ELM which are living as normal flora in human blood and we can't isolate pure cultures of K. sarcoidosis without other types of ELM living as normal flora in human blood.

We believe that different types of ELM have common antigens to M. tuberculosis and M. Bovis. This is the explantation why M. bovis BCG may be used as a vaccine against tuberculosis and why K. sarcoidosis mixed with ELM as normal flora can produce experimental skin sarcoidosis in a human volunteer.

CONCLUSIONS

ELM multiplication is impossible without native human blood. Only irradiated human blood can be used for subcultures because there are always ELM in human blood.

ELM multiplication requires time (days) and this fact explains why most of the erythrocytes in sarcoidosis patients are normal.

Auto-infection experiments are unknown in the sarcoidosis literature and they must be considered carefully by every specialist. The standard strain is preserved for 30 years in NBIMCC 3300, 1995, Kalfinella sarcoidosis sp. novo.

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The Blood of a healthy human is never sterile!

Emil Kalfin

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