

Probiotic Bulletin

A Newsletter for Healthcare Professionals



Human papillomavirus (HPV) & cervical cancer: A new field for probiotic research



Interview with an expert:

Dr Jane Woyka MA MB BCHIR MRCGP

Q Can you tell us about yourself?

A I have been a GP partner at the Harrow Health Care Centre since 1984. I trained in general practice at the Royal Free Hospital after graduating from Cambridge. I am also an Associate Specialist at the menopause clinic at Northwick Park and St Mark's Hospital.

Q So you have a particular interest in women's health?

A Yes, I am a full-time GP with a passionate interest in women's health. I am a fully accredited family planner, a GP Adviser to the Department of Health on Cervical Screening for the last ten years, and involved in promoting the cervical cancer vaccine and advising on human papillomavirus (HPV)-screening implementation. I helped establish special clinics for menopause patients who have cancer, and I am a Council Member of the British Menopause Medical Advisory Committee. I am also fascinated by diet and obesity, and trained as a bariatric physician.

Q How big a problem is cervical cancer in the UK? Are women of any age affected?

A Cervical cancer is the eleventh most common cancer in women in the UK. However in women under 35 years it is the most common cancer.¹

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Q&A

Interview with an expert: (continued)

Q Cervical cancer received a lot of press after the death of Jade Goody. Did this help improve screening rates?

A About half a million extra cervical screenings occurred in England during the period in which she was diagnosed and died. At its peak, screenings were 70% higher than expected. The pattern of increased attendance mirrored the pattern of the media coverage - it is likely this did save a number of lives.

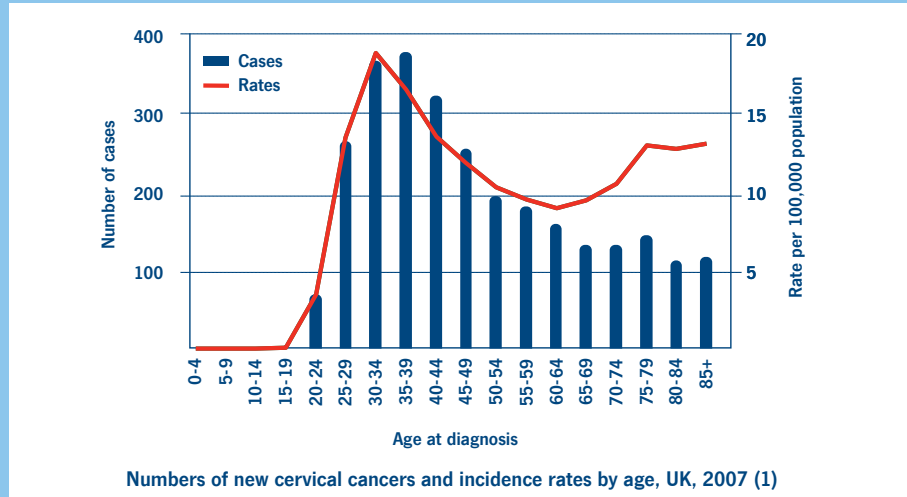
Q Can you explain more about the cervical screening test itself?

A Cervical screening is **not** a test for cancer but it does detect and treat early abnormalities which could lead to cancer if left untreated. It can prevent 75% of cancers developing but it is not a diagnostic test, and may not always detect early cell changes that could lead to cancer. Using a plastic brush, a sample of cells is taken from the neck of the cervix and transferred into a vial of fluid, which is filtered to produce an even layer of cells across the slide (a "liquid based cytology" sample). Cytologists evaluate the slides, grading the levels of change (mild, moderate and severe). Moderate and severe abnormalities are referred for colposcopy and biopsy (the actual diagnostic tests). Low grade abnormalities are now additionally tested for HPV. If the sample is negative for high risk HPV, there is almost no risk of developing cervical cancer in the next ten years. If positive, particularly for high risk strains 16 and 18, the risk is much greater.

Q What are HPV and why are certain types associated with cervical cancer?

A Cervical cancer is caused by HPV, which infects most women at some point in their lives. Of over 130 different types of HPV, around 40 infect the genital area. Some cause genital warts; other types can cause cancer (HPV 16, 18, 31, 33, 35).

The virus is commonly transmitted by close genital contact, not necessarily via full sexual intercourse as it is not transmitted in body fluids. Many people



Numbers of new cervical cancers and incidence rates by age, UK, 2007 (1)

are unaware of infection as it causes no damage initially; in 99% of cases it is detected and killed by the immune system. For the unlucky few, the virus persists to cause progressive damage and transform cells to a pre-cancer state. For about 1% of the population, if undetected, it develops into cervical cancer. Unfortunately we have no way of knowing who. Nearly all of us will come in contact with HPV infection.

Q The cervical cancer vaccine is a preventive treatment. Are there any other medical treatments for HPV infections?

A No there are no medical treatments for HPV infection. The two HPV vaccines available afford 100% protection against HPV 16 and 18, which are responsible for 70% of cervical cancers. Since September 2012, UK schoolgirls have been immunised with Gardasil, which also protects against genital warts (caused by two non-cancer causing strains: 6 and 11). Cervarix, which was used for the first three years, probably offers better cancer protection: cross protection against other cancer-causing strains and possibly better long-term immunity. We believe the immunity generated by the cervical cancer vaccines is lifelong and nine times greater than that generated by naturally-acquired HPV infection, which may not be for life.

Q What did you think of this recent study (see page 3) looking at effects on HPV and cervical abnormalities?

A This study is very interesting. Obviously this is a pilot and involved very small numbers so in a further study it would be good to see random allocation of patients and proper blinding.

It is tempting, however, to be excited at the results, which showed that half of the participants who took the probiotic had resolved their low grade cytological abnormalities after six months in comparison to just under 30% in the non-probiotic group.

The results for HPV clearance after six months were not quite statistically significant, although you would have expected HPV clearance rates and resolution of low grade abnormalities to be very similar.

Q Do you think the results are worthy of further exploration, i.e. a bigger placebo-controlled study?

A It would have been good to follow these patients for two years as we know that most HPV infection clears within about two years. It would also have been good to know if at two years, the probiotic group had better results for both HPV clearance and resolution of cytological abnormalities.

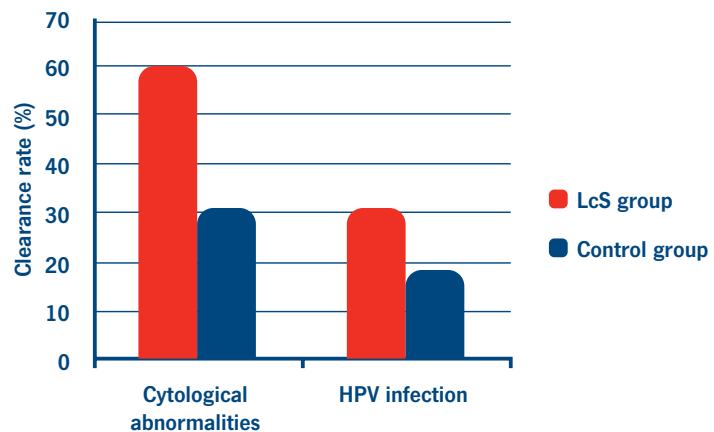
As we have identified that almost everybody sexually intimate acquires HPV infection, it would be crucially helpful to know or identify those individuals who do not clear the HPV. It would be marvellous to think that by taking a probiotic we could reduce the risk of developing cervical cancer, particularly in those with persistent HPV infections. At the moment we have absolutely no way of treating HPV other than advising patients to stop smoking.

Q Can you speculate on the possible mechanism of activity?

A There are obviously lots of theories as to how probiotics may influence disease processes in the gut, in the bladder

Verhoeven V et al (2012) Probiotics enhance the clearance of human papillomavirus-related cervical lesions: a prospective controlled pilot study. *European Journal of Cancer Prevention* 22:46-51.

In this open-label prospective controlled pilot study at Antwerp University in Belgium, 54 women diagnosed with low-grade squamous intraepithelial lesion associated with human papillomavirus (HPV) were monitored for six months. Over this time, the test group consumed a daily probiotic (*Lactobacillus casei* Shirota fermented milk drink) and the control group did not consume any probiotic. Probiotic effect was assessed by conducting a cervical test (PAP smear) and investigating HPV status after six months. Results are shown in the graph (below).



HPV-positive women: effect of probiotic intervention

and in the urogenital tract. It's a bit of a minefield. Rather than speculate on mechanisms, I'd rather just observe that there did seem to be a significant positive influence in the probiotic group.

My experience with probiotics is that they never have any adverse effect and

are readily taken by patients who often believe they help them return to good health. I hope there will be a further trial exploring the effect of probiotics in resolution of HPV infection.

Reference list is available from science@yakult.co.uk or at www.yakult.co.uk/hcp.

Did you know...

- Worldwide, cervical cancer is the third most common cancer in women and fourth most common cause of cancer-related death ¹
- In 2008, 958 women died of cervical cancer in the UK ²
- In 2009, 2,947 cases of cervical cancer were diagnosed in England ²
- 40% of cervical cancer cases are diagnosed in women aged between 30 and early 40s ³
- 10% of 16-year old girls are infected with the cancer-causing HPV, rising to 40% for 24-year olds ⁴
- Half a million women still do not attend their screening every year in the UK. Despite this, the screening program is estimated to save approximately 4,500 lives every year ⁵

Probiotic use for an antibiotic-resistant opportunist pathogen

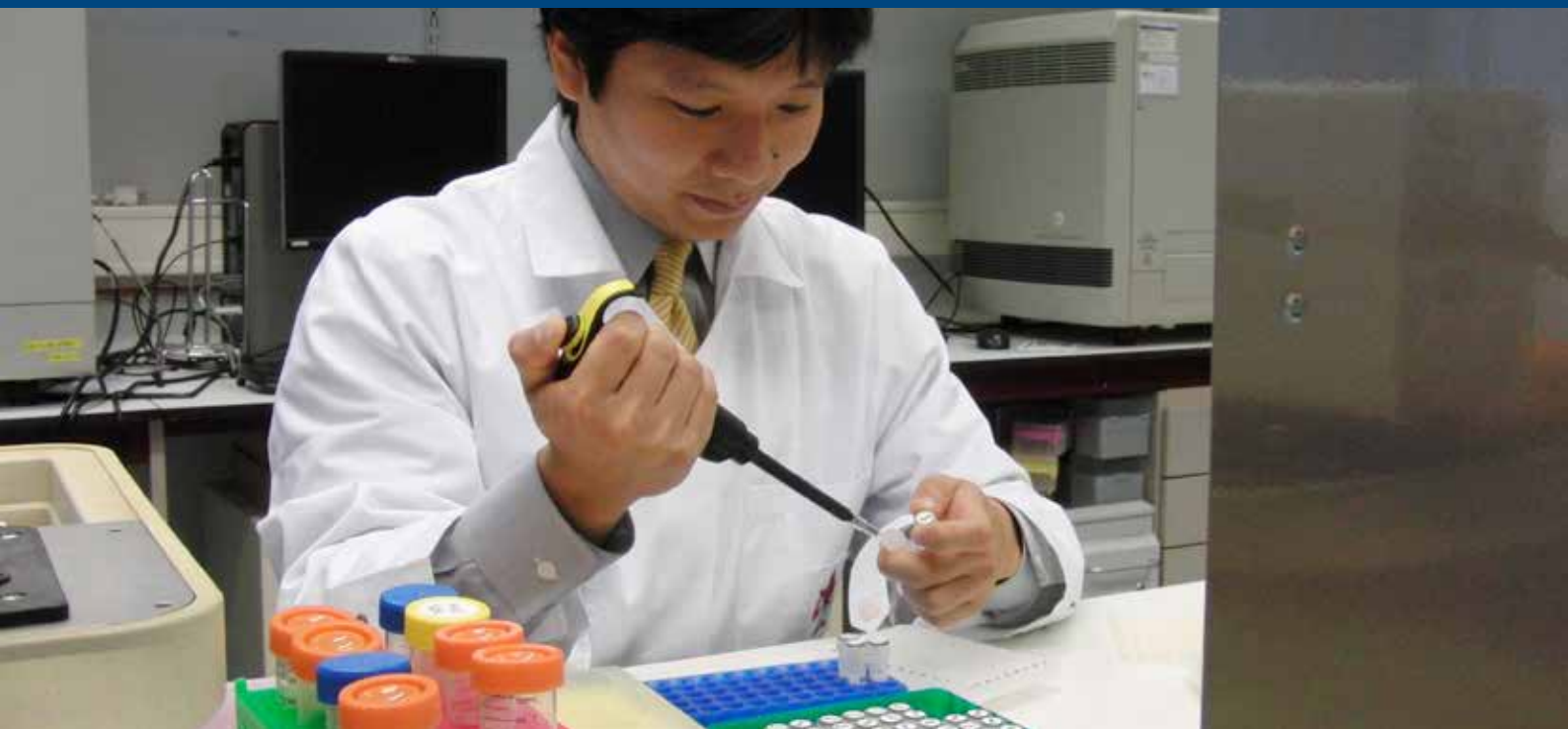
The majority of fatalities in burns patients are due to infected wounds, which are a risk until healing is complete. The danger of infection is now even more serious with the spread of multiple antibiotic-resistant organisms in hospitals. A recent UK survey of burns patients found multi-organ failure to be the primary cause of death with sepsis the primary trigger (51%); the nosocomial pathogen *Pseudomonas* was the most common isolate.

Unfortunately, such species have a high level of intrinsic drug resistance and are becoming resistant to even more antibiotics, including carbapenems. These β -lactam antibiotics are resistant to most β -lactamases, thus are often used as 'last resort' antibiotics for Gram-negative infections. This is why the emergence of metallo β -lactamase (MBL) strains able to hydrolyse is such a great concern. The first plasmid-mediated MBL was reported in *P. aeruginosa* in Japan in 1991; MBL strains have now been reported across the world, including in Europe.

This article describes a patient with serious burns that became infected with an extremely drug resistant (XDR) MBL *P. aeruginosa*, an infection that seemed to contribute to wound breakdown and failure to heal. As the strain was also found in the patient's gut, this was probably why the wounds became re-contaminated over a five month period. A dietitian in the hospital finally suggested trying a daily probiotic (*L. casei* Shirota) to see if this influenced the gut microbiota to prevent carriage of the pathogen. After two weeks on the probiotic, the XDR strain was no longer detected (only drug-sensitive *P. aeruginosa* were isolated). This change persisted until the patient was discharged, 15 months after first suffering the burns.

Thomson CH et al (2012) Yakult: a role in combating multi-drug resistant *Pseudomonas aeruginosa*? *J Wound Care* **22**(11):566-569.

Reference list is available from science@yakult.co.uk or at www.yakult.co.uk/hcp.



| An Expert's View

Catching up with Dr Hiroshi Makino

Q What do you do?

A I'm a microbiologist, and an associate senior researcher at Yakult's research centre in Belgium.

Q Who is your favourite scientist? And why?

A Not sure I can decide on just one. When I was younger, I was fascinated by Dr Hideyo Noguchi, a Japanese bacteriologist who discovered *Treponema pallidum*, the causative agent of syphilis. He spent his whole life investigating virus diseases in order to save lives around the world (he died of yellow fever, contracted doing this). He triggered my interest in human health. I mustn't forget to mention Dr Shinya Yamanaka, who has just been awarded the Nobel Prize in Physiology or Medicine for his discovery that mature cells can be converted to stem cells. It's nice to hear great news of fellow countrymen; it inspires us.

Q Which areas of research interest you?

A I'm interested in the development of the human microbiota because this is so important for health - not surprising as 70% or more of the immune system is in the GI tract. Interaction with the gut microbiota is key to the development and proper functioning of the immune system.

The gut is assumed to be sterile at birth. Microbial colonisation during infancy is particularly critical as this can have a lifetime effect.

Q How do probiotics and the microbiota fit into that?

A Bifidobacteria are thought to help development of the neonatal mucosal immune system. Our data suggest that transfer of the mother's bifidobacteria to her baby is important in determining early colonisation by these species, so having a healthy intestinal microbiota during pregnancy may benefit the baby (Makino *et al* 2011). I believe that mothers should be aware of the relationship between their own microbiota and that of their babies, and how this may influence their child's immune system, possibly with long term (even lifelong) health effects.

Q What does your research involve?

A Using some of the techniques developed by Yakult, I'm analysing the microbiota of infants, trying to identify which factors affect its development, particularly those associated with long term benefit. This might help in developing new generation nutritional concepts for infants.

We are comparing the microbiota of breast-fed babies with formula-fed babies, and also babies born by C-section born with those born vaginally. So far, we see several differences in their microbiota development in terms of species, numbers, and when they start to colonise. But we need to do a lot more work to understand the health implications of this.

Q When you are not engrossed in research what are your hobbies?

A I like to play any kind of sport (tennis, swimming, biking, skiing...). Whenever I have some time off, I like to travel around the world and visit world heritage sites.

Makino H *et al* (2011) Transmission of intestinal *Bifidobacterium longum* subsp. *longum* strains from mother to infant, determined by multilocus sequencing typing and amplified fragment length polymorphism. *Appl Environ Microbiol* **77(19)**:6788-6793



Microbiology in Schools Advisory Committee Annual Competition 2012: sponsored by Yakult

'Fit for purpose: microbes and healthy living' was the topic for the 24th Annual MiSAC competition for the best one-page, illustrated news article for a healthy living magazine aimed at improving understanding of the contribution of the activities of microbes to healthy living.

More than 60 group entries from 55 schools and colleges yielded over 500 separate entries consisting of some 250 from Key Stage 3 and 160 from KS4. Overall, nearly 500 students

were involved from England, Wales, Scotland, Northern Ireland and Ireland, and from British schools in Belgium and Germany.

Members of MiSAC were joined on the judging panel by Dr Linda Thomas and Leanne Hewitt from Yakult. Of the suggested topics, 'mycoprotein' and 'the normal flora of the gut' generated the most interest, as well as 'modification of the normal gut flora (probiotics, prebiotics)'. Appropriately illustrated and attention-grabbing layouts

found favour as did evidence of good science and use of an entrant's own words. The requirement to give a name to the magazine, a title to the article and sources of further information provided an outlet for imaginative and witty talents. The results list and winning entries can be viewed on the MiSAC Annual Competition webpage (www.misac.org.uk) and on the Awards section of Yakult's website (www.yakult.co.uk/hcp).

Dr John Grainger (Chairman of MiSAC)

Yakult... to boldly go where no probiotic has gone before! (apologies to Captain Kirk)

Space – the final (probiotic) frontier. Yakult's mission, to... OK, enough with the Star Trek.

In collaboration with the Japan Aerospace Exploration Agency, Yakult HQ has announced their involvement in an initiative to set up research studies on the International Space Station, studying the potential of probiotics for astronauts.



Astronauts experience physical and psychological stress, not just during take-off and landing, but because of their sometimes prolonged periods in zero-gravity and confined environments, such as in the International Space Station. Spaceflight even within Earth's gravitational field can affect the immune response and extended space flights have the added risk of exposure to solar particle event radiation. Could immunosuppression during spaceflight prevent us colonising beyond our own planet?

Illness is a real concern: infections could arise from the astronaut's endogenous and commensal microbiota (which might include opportunistic pathogens and latent viruses) as well as microbial contaminants in the space capsule. A further issue has also been identified: microgravity may increase the virulence of certain pathogens. Other lines of microbiological research relating to space travel include:

- **Sterility:** keeping extra-terrestrial samples free from human or environmental contamination; limiting cross contamination during missions; decontamination procedures against any extra-terrestrial microbes;
- **Pathogens:** effects on virulence, growth, latency and biofilm formation; detection on surfaces;
- **Immune response:** stress-induced changes;
- **Intestinal microbiota:** changes during short and long term missions;
- **Life support systems:** setting up diverse and stable microbial communities for colonisation outside the Earth.

So watch this 'space' for updates (apologies again...)

Reference list is available from science@yakult.co.uk or at www.yakult.co.uk/hcp.

Probiotics and management of diarrhoea in HIV

Diarrhoea is often suffered by HIV patients and ways to minimise this are needed. In this critical appraisal of the evidence, the main medical databases were searched for randomised, controlled trials using probiotics in these patients, with diarrhoea as an endpoint. Only four relevant trials were found so no strong conclusions could be made, but it was commented that probiotics could be an option due to their safety and lack of drug interaction. Guinane S (2012) The effectiveness of probiotics for managing diarrhoea in people with HIV infection: a critically appraised topic. *HIV Med* Sept 19 [Epub ahead of print].

Clinical review: irritable bowel disease

This extensive review focused on the occurrence, pathophysiology and classification of IBS symptoms, and the potential usefulness of probiotics. For example, there could be symptom improvement due to any anti-inflammatory effects, and there is also some evidence that certain strains may help abdominal pain and bloating. It was concluded that probiotics were superior to placebo but that it was difficult to ascertain which strains were beneficial due to the heterogeneity of the trials.

Ford AC & Talley NJ (2012) Irritable Bowel Syndrome. *BMJ* **345**:37-42.

Lactose intolerance: probiotic study

In this study of people with lactose intolerance (n=27), consumption of two probiotic strains (*Lactobacillus casei* Shirota, *Bifidobacterium breve* Yakult) over a four week period was associated with improved symptoms and a decrease in breath hydrogen after lactose intake. These effects continued for more than three months after probiotic consumption ceased.

Almeida CC et al (2012) Beneficial effects of long-term consumption of a probiotic combination of *Lactobacillus casei* Shirota and *Bifidobacterium breve* Yakult may persist after suspension of therapy in lactose-intolerant patients. *Nutr Clin Pract* **27**(2):247-51.

Probiotics in the critically ill

Databases from 1980 to 2011 were searched for placebo-controlled, randomised trials with critically ill patients, examining outcomes such as infection, mortality and length of stay. From the 23 RCT identified, it was concluded that probiotics appear to reduce infectious complications, including ventilator-associated pneumonia, and also influence ICU mortality.

Petrof EO et al (2012) Probiotics in the critically ill: A systematic review of the randomized trial evidence. *Crit Care Med* **40**(12):3290-3302.

Lactobacilli do not contribute to obesity

This corrects misconceptions that probiotics might promote human obesity, which arose from observations of enhanced growth in livestock and children. This was most likely due to probiotic reduction of disease risk and promotion of normal growth, not promotion of adipose tissue. Recent animal studies were highlighted that, conversely, indicate probiotics may contribute to weight loss and help reduce risk of obesity-related disease.

Lahtinen SJ et al (2012) *Lactobacillus* species causing obesity in humans: where is the evidence? *Beneficial Microbes* **3**(3):171-174.

Gut Microbes and Health. *Nature Insight* (2012) **489(7415):219-257.**

Over the past few years, there have been numerous high level research publications on the human gut microbiota, including several from the US Human Microbiome Project and the European Metagenomics of the Human Intestinal Tract group. This Yakult-sponsored supplement, which can be downloaded or ordered free from Nature's website, provides clear and comprehensive summaries of the new findings, accompanied by some excellent diagrams.

In the accompanying sponsor feature, scientists from Yakult's institute in Japan (Umesaki et al) describe their own research, including the development of the YIF-SCAN, a state of the art microbial analysis system.

In this supplement:

- **Gut microbes and health. Editorial**, Lupp C, Skipper M, Weiss M.
- **Diversity, stability and resilience of the human gut microbiota**, Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R.
- **Reciprocal interactions of the intestinal microbiota and immune system**, Maynard CL, Elson CO, Hatton RD, Weaver CT.
- **Functional interactions between the gut microbiota and host metabolism**, Tremaroli V & Backhed F.
- **Genomic approaches to studying the human microbiota**, Weinstock G.



STOP PRESS!

Hot on the heels of London 2012, Yakult's International Symposium - London 2013

'The Intestinal Microbiota and Probiotics: Exploiting their Influence on Health'
The Queen Elizabeth II Conference Centre, London
Monday 22 and Tuesday 23 April 2013.

Visit www.yakultsymposium.com for programme information and to register. Places are limited and early bird rates apply. Approved by the Society of Biology for purposes of CPD (18 credits), and by the Institute of Biomedical Science (10 credits). If you are submitting a poster, contact science@yakult.co.uk (we may cover your registration fee).

STOP PRESS!

Find the science behind Yakult at our new website for healthcare professionals www.yakult.co.uk/hcp

- An up-to-date and searchable library of *L. casei* Shirota research papers.
 - A comprehensive source of probiotic information.
- Free educational resources, including pocket sized Bristol Stool Scale cards.

Contact science@yakult.co.uk for:

- ✓ Free educational talks for your department
- ✓ Advice on probiotics
- ✓ Newsletter signup
- ✓ Free trial period of Yakult (subject to discussion)

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