

# Clinical safety issues of measles, mumps and rubella vaccines

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The clinical safety of measles and measles–mumps–rubella vaccines has been questioned in recent reports that propose a possible link between measles virus or measles vaccines and the occurrence of juvenile Crohn disease and autism. This article reviews the outcomes of several laboratory investigations which were carried out independently to identify the presence or absence of measles virus in the intestinal tissues derived from cases of inflammatory bowel disease. One research group reported the presence of measles virus particles and genomic RNA in inflammatory bowel disease tissues, but this could not be confirmed by other groups, despite use of techniques that are highly specific and sensitive for the detection of measles virus nucleic acid in clinical specimens down to the molecular level. Based on the published data reviewed here, it can be concluded that there is no direct association between measles virus or measles vaccines and the development of Crohn disease, a conclusion which is supported by most epidemiological findings.

**Keywords:** measles vaccine, adverse effects; mumps vaccine, adverse effects; rubella vaccine, adverse effects; vaccines, attenuated, adverse effects and genetics.

*Voir page 202 le résumé en français. En la página 202 figura un resumen en español.*

## Introduction

Some concerns have recently been expressed on the post-immunization safety of live attenuated measles vaccine and measles–mumps–rubella (MMR) vaccine because of reports which have proposed a possible link between measles virus vaccines or other paramyxovirus infections and the establishment of juvenile autism, Crohn disease (CD) and other forms of inflammatory bowel disease (IBD) (1–4). This hypothesis was based on observations which showed that wild measles infection at the neonatal stage could increase the risk of development of CD late in life (5, 6). The present review discusses the findings of experimental work carried out at the National Institute for Biological Standards and Control (NIBSC) and in other laboratories to identify the presence or absence of measles virus RNA in clinical tissues derived from IBD cases. In addition, some information on the virological components of MMR vaccine and tests carried out on them prior to release for human use is provided.

## Virological background to MMR vaccine

Vaccines against measles, mumps and rubella are produced from live attenuated viruses which have

been propagated in a variety of cell substrates, including embryonated chicken eggs and/or human diploid cells. Each component of MMR vaccine is initially prepared in the monovalent form, each of which is then mixed together to produce a trivalent form in which the component virus population is present in a well-defined quantity sufficient to induce an effective immune response in a vaccine recipient. In addition to the trivalent form, commercial preparations are also available in the monovalent form.

The genomes of all three viruses consist of a single-stranded RNA molecule that has negative polarity for measles and mumps and positive polarity for rubella. The genetic organization of various genes within the linear genome has been fully mapped and their sequence determined completely for a number of strains. However, the structure–function relationships of the viral genes, their protein products, and host–virus interactions at the nucleic acid and protein levels remain to be elucidated. There is a lack of understanding, at the molecular level, about the mechanism(s) of virus attenuation during multiple passages in embryonated eggs and cells derived from them or other sources. At present, the genetic domains that could be used as molecular markers to confirm whether the virus population present in the commercial formulations of MMR vaccines is effectively attenuated, or not, remain to be identified. These domains have been mapped and characterized at the molecular level for poliovirus (7) and their analysis provides good indicators about the neurovirulence potentials of vaccine lots before they are released for human use (8). The current regulatory requirements of MMR vaccine production demand a comprehensive evaluation of each candidate vaccine

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seed material for its virological, serological, and clinical safety, including evidence for the absence of neurovirulence in susceptible animals. The seed that passes the regulatory requirements is then used as the inoculum to generate vaccine production lots under standard manufacturing conditions. Product quality is maintained by ensuring production consistency between vaccine batches generated from the same seed. It is believed that at the molecular level the genomes of measles, mumps and rubella viruses are sufficiently stable, as there are no reports indicating the occurrence of genetic recombination. This is in contrast to the situation for poliovirus, the genetic stability of which is difficult to maintain because of RNA recombination and also the rapid reversion of the attenuating mutations within the genome. In the United Kingdom, every batch of MMR vaccine is routinely tested, prior to release, for its potency, thermostability and identity of its viral components using several virological and serological assays, and the product records are periodically reviewed. Only vaccine batches meeting the required specifications are allowed to be released for immunization use. The cumulative data (not shown) produced at NIBSC over the years suggest a good level of consistency between vaccine batches in relation to all tested parameters. Other procedures to evaluate and to monitor the consistency of MMR vaccine production at the cellular and molecular levels are in the development stage at NIBSC.

## Clinical issues

MMR vaccines currently licensed in the United Kingdom meet all the regulatory and control requirements that are essential to demonstrate their clinical efficacy and safety in the vaccine recipients. However, in the past, cases of aseptic meningitis following MMR immunization have been reported in some children, especially those receiving the vaccine containing the Urabe strain of mumps virus (9, 10). This led in 1992 to the withdrawal from immunization schedules of mumps vaccine containing this strain. Mumps vaccines currently licensed in the USA, Canada, United Kingdom, and several other European countries contain the Jeryl-Lynn vaccine strain of mumps virus. The clinical safety and immunogenicity of this strain is well established since millions of vaccine doses have been used worldwide without any reports of serious adverse effects.

Rubella vaccines produced by all major vaccine producers contain RA27/3 vaccine strain, which is sufficiently immunogenic but less reactogenic than the previously used HPV77 or Cendehill vaccine strains. Rubella vaccine is extremely effective in reducing the incidence of congenital rubella syndrome (CRS) in the United Kingdom (11), where the rubella/MMR vaccination campaign has been vigorously implemented. Rubella vaccine is well tolerated by most recipients; however, there are reports which suggest a link between rubella immunization and

acute arthritis in some adult women (12). The incidence of such arthritis is substantially lower than that observed after natural rubella virus infection. Other studies reported recently, however, failed to identify any significant association between chronic arthropathy and rubella vaccination among women (13–16).

The key vaccine safety issue has been the recent suggestion that measles and MMR vaccines, derived from live attenuated viruses, can lead to the development of juvenile Crohn disease and autism (1–3). The adverse publicity on this issue resulted in a decline of MMR vaccine coverage in the United Kingdom (17, 18), leading to fears of measles, mumps and rubella epidemics. The proposed link between measles and Crohn disease mainly emerged from earlier epidemiological and case-control studies, which claimed a temporal relationship between the rising numbers of Crohn disease cases and the introduction in 1968 of live attenuated measles virus vaccine in the National Immunisation Schedule in the United Kingdom (1, 3). However, other reports suggest that the observed rise in the number of IBD cases started much earlier than the introduction of the measles vaccination campaign (19–21), supporting the views that other factors may have been involved in contributing to the reported rise in IBD incidence in the United Kingdom. In addition to the epidemiological evidence, the physical presence of measles virus in clinical tissues obtained from cases of Crohn disease and ulcerative colitis has been reported (22, 23). The experimental approach for the demonstration of measles virus in IBD tissues was based on the examination of the material by transmission electron microscopy, immunocytochemistry and in-situ hybridization, using a gene probe specific for the nucleocapsid (N) gene of the measles virus genome (22). In the past, these approaches have been shown to be misleading, especially when applied at high sensitivity (24). The specificity of the reagents and methodology used in the original study, which showed the presence of measles virus in the intestinal tissues of IBD cases (22), have been questioned on the grounds that the paramyxovirus nucleocapsid-like particles resemble normal cellular structures (25), while the antibody used for immunostaining has been reported to show non-specific reactivity with the cellular protein(s) (26). Previously, a similar conclusion about the nonspecific activity of the measles antibody was drawn from the immunohistological study that used measles anti-matrix (M) protein antibody to identify measles virus in IBD tissues (27).

It was proposed that the examination of IBD tissues by measles-specific polymerase chain reaction (PCR) could provide a definitive answer to these uncertainties (24, 25), because this technique should amplify measles virus RNA sufficiently to permit its unambiguous molecular characterization. Several groups adapted and applied the PCR-based approach to detect the presence of measles virus genomic RNA in tissues derived from IBD and non-IBD cases (28–32). The findings of these investigations, conducted

independently in different laboratories, are summarized in Table 1. The sensitivity limits of the established reverse transcription (RT)-PCR assays varied considerably between laboratories.

The Royal Free Hospital group established a method for enriching measles virus RNA molecules prior to RT-PCR amplification, by oligonucleotide hybrid capturing to improve the assay sensitivity. Using this approach, they examined several specimens of tissue from patients with Crohn disease, ulcerative colitis, sigmoid volvulus and indeterminate colitis by targeting the N and/or H gene portions of the measles virus genome. The assay sensitivity limits determined with the in-vitro-derived measles virus RNA transcripts were found to be around  $10^4$  genomic molecules per assay (32).

The NIBSC group also developed a highly sensitive measles-virus-specific molecular diagnostic system by performing the reverse transcription and primary PCR amplification with GeneAmp EZ *rTth* DNA polymerase (Perkin Elmer) in a single tube followed by nested PCR amplification separately with AmpliTaq DNA polymerase (Perkin Elmer). We examined several preparations of peripheral blood lymphocytes and colonoscopic biopsies of Crohn disease, ulcerative colitis and indeterminate colitis to identify the presence of measles virus RNA in them. This approach could amplify the measles virus target RNA templates from control samples originally containing the virus particles at ca.  $5.5 \times 10^{-3}$  plaque-forming units per reaction, but no IBD sample was found positive. In terms of clinical material this method was able to amplify the measles virus genomic sequence successfully from a nucleic acid mixture extracted from the specimen equivalent to about 18 cells of subacute sclerosing panencephalitis (SSPE) brain material (31). Experiments designed to quantitate the IBD clinical tissues used for RT-PCR-nested PCR examination, in relation to a housekeeping gene (human  $\beta$ -actin) DNA products,

revealed that in each reaction the nucleic acid extracted from about one million cells was examined for the presence of measles virus genome, is approximately 50 000-fold more than the SSPE material required to produce a measles-specific positive signal under identical experimental conditions (30, 31).

The PCR diagnostic assays developed by the two Japanese groups were sensitive and specific for the measles virus genome identification (28, 29). One group established a system that could detect the presence of measles virus RNA templates in a reaction mixture originally containing only a single copy of the genome (29). Both groups examined several intestinal tissues, taken surgically or through endoscopic biopsies, by targeting several regions of the measles virus genome for amplification (28, 29).

Despite applying the most sensitive molecular technology currently available, none of the groups, including the group at the Royal Free Hospital, who originally formulated the measles-IBD hypothesis, has been able to demonstrate the presence of measles virus genome sequence in gut tissues taken from IBD cases either surgically or collected through colonoscopic biopsy procedures (28–30, 32). The only measles-virus-specific signals observed from the IBD tissue extracts have been reported to be produced through cross-contamination of samples with the experimental controls (31, 32). The fact that all RT-PCR-based studies reached the same conclusion, despite using different experimental approaches and targeting multiple regions of the genome, is a clear indication that the failure to detect the measles virus RNA molecules in IBD and non-IBD tissues is due to their complete absence rather than their presence in a very low copy number (3, 24, 32, 33).

Recent serological and epidemiological studies also show the lack of association between measles or MMR vaccines and the development of IBD and autism (21, 34, 35). However, there are reports

Table 1. Summary of the findings of experimental studies performed to identify measles virus in IBD tissues<sup>a</sup>

| Study group                          | IBD form    | Tissue source                | Experimental procedure                           | Assay sensitivity        | MV presence | Reference |
|--------------------------------------|-------------|------------------------------|--|--------------------------|-------------|-----------|
| Royal Free Hospital, London, England | CD, UC      | Resected material            | TEM, immunohistochemistry, in situ hybridization | Not determined           | Positive    | 22, 23    |
| Akita & Osaka University, Japan      | CD, UC      | Resected + biopsy samples    | N, M, F, H gene-specific RT-PCR                  | Not determined           | Negative    | 28        |
| Hirosaki University, Japan           | CD, UC      | Resected material            | N, F gene-specific RT-PCR-nested PCR             | Single genome copy       | Negative    | 29        |
| NIBSC, England                       | CD, UC, IDC | Lymphocytes + biopsy samples | N gene-specific RT-PCR-nested PCR                | $5.5 \times 10^{-3}$ PFU | Negative    | 30, 31    |
| Royal Free Hospital, London, England | D, UC, IDC  | Resected + PBMC              | N, H gene-specific RT-PCR                        | $10^4$ RNA molecules     | Negative    | 32        |

<sup>a</sup> MV = measles virus; IBD = inflammatory bowel disease; CD = Crohn disease; UC = ulcerative colitis; IDC = indeterminate colitis; PBMC = peripheral blood mononuclear cells; TEM = transmission electron microscopy; PFU = plaque-forming unit; N = nucleocapsid protein gene; M = matrix protein gene; F = fusion protein gene; H = haemagglutinin protein gene.

which indicate a continuous rise in the incidence of cases of paediatric Crohn disease (36, 37), the reasons for which are unknown although it could be attributed to improved methods of diagnosis.

Long-term persistence of measles virus is possible in several human tissues including the brain, lung, liver, spleen and kidney, and the molecular diagnostic approaches (i.e. RT-PCR-based), when

applied meticulously, could produce evidence for the presence of the virus in clinical tissues where it genuinely existed (38, 39). The data reviewed here support continuing with measles, mumps and rubella immunization, using the licensed commercial vaccines, to reduce the risks of several life-threatening and debilitating infections associated with these three viruses. ■

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## Résumé

### Innocuité des vaccins antirougeoleux/antiourlien/antirubéoleux

Des rapports ont récemment fait état de préoccupations relatives à l'innocuité clinique du vaccin antirougeoleux et du vaccin antirougeoleux/antiourlien/antirubéoleux (ROR), rapports qui évoquent un lien éventuel entre le virus rougeoleux ou les vaccins antirougeoleux et la survenue d'une maladie de Crohn et d'un autisme juvéniles. Cet article passe en revue les résultats de plusieurs études de laboratoire effectuées indépendamment afin de rechercher la présence ou l'absence du virus rougeoleux dans les tissus intestinaux provenant de cas d'infection inflammatoire du tube digestif (« inflammatory bowel disease (IBD) » des Anglo-Saxons). La présence du virus rougeoleux dans ces tissus a été signalée par un groupe ayant utilisé des méthodes conventionnelles, mais n'a pas pu être confirmée par les autres.

On y trouve également des informations sur les constituants virologiques des vaccins ROR et sur les tests de contrôle effectués avant leur mise en circulation pour un usage médical. A l'heure actuelle, les domaines génétiques qui pourraient être utilisés comme marqueurs moléculaires servant à confirmer si la population virale présente dans les formulations commerciales des vaccins ROR est efficacement atténuée ou non ne sont toujours pas identifiés. Ces domaines ont été cartographiés et caractérisés à l'échelle moléculaire pour le poliovirus et leur analyse fournit de bons indicateurs du potentiel de neurovirulence des lots de vaccins avant qu'ils soient mis en circulation pour un usage chez l'homme. Dans la réglementation actuelle, les normes de production du vaccin ROR exigent une évaluation complète de tous les matériels de semence des vaccins candidats, dont l'innocuité virologique, sérologique et clinique doit être attestée et qui doivent faire la preuve de l'absence de neurovirulence chez des animaux sensibles. Le virus de la semence qui satisfait aux normes est ensuite utilisé comme inoculum pour produire des lots de vaccins selon des normes de fabrication standard. La qualité des produits est assurée par l'uniformité des lots de vaccin issus d'un même lot de semence. Au Royaume-Uni, chaque lot de vaccin ROR – avant sa mise en circulation – est soumis en routine à des tests d'activité, de

thermostabilité et d'identité du constituant viral au moyen de plusieurs dosages virologiques et sérologiques, et les dossiers du produit sont périodiquement examinés. Seuls les lots de vaccin qui satisfont aux normes peuvent être mis en circulation pour la vaccination.

Les vaccins ROR actuellement autorisés sur le marché au Royaume-Uni satisfont à toutes les normes réglementaires et de contrôle indispensables pour attester leur efficacité et leur sécurité cliniques chez les receveurs. Cependant, on a signalé dans le passé des cas de méningite à liquide clair faisant suite à une vaccination par le ROR chez certains enfants, en particulier chez ceux qui avaient reçu le vaccin contenant la souche Urabe du virus ourlien. Tous les autres vaccins antiourliens ayant reçu une autorisation de mise sur le marché sont cliniquement sûrs. Les vaccins antirubéoleux actuellement produits par tous les grands fabricants de vaccins contiennent la souche vaccinale RA27/3, qui est suffisamment immunogène mais moins réactogène que les souches HPV77 ou Cendlehill utilisées auparavant. Le vaccin antirubéoleux est bien toléré par la plupart des sujets, mais on a signalé chez certaines femmes adultes des cas où il pourrait y avoir un lien de cause à effet entre la vaccination antirubéoleuse et une arthrite aiguë; cependant, leur incidence est sensiblement inférieure à celle observée après une infection naturelle par le virus de la rubéole.

Le principal sujet évoqué dans cette analyse est l'application de la PCR (« polymerase chain reaction ») pour rechercher la présence ou l'absence de particules du virus rougeoleux dans les tissus cliniques provenant de cas de maladie de Crohn, de rectocolite hémorragique, de colite indéterminée et autres échantillons cliniques. Aucune des études menées avec la PCR n'a montré la persistance de particules du virus rougeoleux dans les tissus malades. D'après les données publiées examinées dans cet article, on peut en conclure qu'il n'y a pas d'association directe entre le virus rougeoleux ou les vaccins antirougeoleux et la survenue d'une maladie de Crohn, conclusion que viennent appuyer la plupart des résultats épidémiologiques.

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## Resumen

### Seguridad clínica de las vacunas contra el sarampión-parotiditis-rubéola (SPR)

En los últimos tiempos se ha expresado preocupación por la seguridad clínica de las vacunas SPR en informes que

sugieren una posible relación entre el virus del sarampión o las vacunas antisarampiónicas y la incidencia de la

enfermedad de Crohn juvenil y el autismo. En este artículo se analizan los resultados de varias investigaciones de laboratorio independientes realizadas para detectar el virus del sarampión en muestras de tejido intestinal de casos de enfermedad intestinal inflamatoria. La presencia del virus del sarampión en dichos tejidos ha sido notificada por un grupo que utilizó métodos convencionales, pero no ha podido ser confirmada por otros.

También se facilita información sobre los componentes víricos de las vacunas SPR y las pruebas de control realizadas antes de su autorización para uso humano. En la actualidad quedan por identificar los dominios genéticos que podrían utilizarse como marcadores moleculares para confirmar si la población de virus presente en las preparaciones comerciales de las vacunas SPR está efectivamente atenuada. Estos dominios se han cartografiado y caracterizado a nivel molecular para el poliovirus, y su análisis proporciona indicadores fiables de la posible neurovirulencia de los lotes de vacunas antes de su autorización para uso humano. Las actuales prescripciones reglamentarias para la elaboración de vacunas SPR exigen una detallada evaluación del material de siembra de las vacunas experimentales para garantizar la seguridad virológica, serológica y clínica de las mismas, incluidas pruebas de la ausencia de neurovirulencia en animales susceptibles. La simiente que supera los requisitos normativos se utiliza como inóculo para generar lotes de producción de vacunas conforme a condiciones de fabricación normalizadas. La calidad del producto se mantiene asegurando la uniformidad de los lotes de vacuna elaborados a partir de la misma simiente. En el Reino Unido se examinan sistemáticamente la potencia, la termoestabilidad y los componentes víricos de todos los lotes de vacuna SPR antes de su autorización mediante diversos análisis virológicos y serológicos, y se realizan exámenes periódicos de los registros del producto. Sólo se

autorizan para fines de inmunización los lotes que cumplen las condiciones exigidas.

Actualmente las vacunas SPR autorizadas en el Reino Unido satisfacen todas las prescripciones reglamentarias y de control esenciales para demostrar su eficacia y seguridad clínica en los receptores de las vacunas. Sin embargo, hay antecedentes de notificación de la aparición de meningitis aséptica postinmunización SPR en algunos niños, especialmente en los que recibieron la vacuna que contenía la cepa Urabe del virus de la parotiditis. El resto de las vacunas anti-parotídicas autorizadas son clínicamente seguras. Las vacunas contra la rubéola elaboradas actualmente por los principales fabricantes de vacunas contienen la cepa vacunal RA27/3, que es suficientemente inmunógena, pero menos reactógena que las anteriores cepas vacunales HPV77 o Cendlehill. La vacuna antirrubéolica es bien tolerada en la mayoría de los casos, pero algunos informes sugieren una relación causal entre la inmunización contra la rubéola y la artritis aguda en algunas mujeres adultas; sin embargo, la incidencia es considerablemente inferior a la observada tras la infección natural por el virus de la rubéola.

El tema principal de esta revisión es la aplicación de la tecnología de la reacción en cadena de la polimerasa (RCP) para determinar la presencia o ausencia de partículas del virus del sarampión en tejidos procedentes de enfermos de Crohn, colitis ulcerosa o colitis de origen indeterminado y en otras muestras clínicas. Ninguno de los estudios basados en la RCP han revelado la persistencia de partículas del virus del sarampión en tejidos afectados por la enfermedad intestinal inflamatoria. Según los datos publicados analizados en este artículo, cabe concluir que no existe una relación directa entre el virus del sarampión o las vacunas antisarampionosas y el desarrollo de la enfermedad de Crohn, conclusión corroborada por la mayoría de los estudios epidemiológicos.

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