Cross-protection from human papillomavirus 16/18 against types 45 and 31: fact or fancy?

‘The holy grail of HPV vaccine development is to create a vaccine that is not limited by type specificity, but instead is able to recognize and neutralize the entire spectrum of HPV types.’

Human papillomavirus (HPV) types 16 and 18 are responsible for approximately 70% of all cervical cancers, followed by HPV types 45 and 31, which represent an additional 10%. In total, there are 15 HPV types that have been associated with the development of cervical cancer [1,2]. HPV vaccination has been shown to be highly effective in preventing precancerous cervical lesions in several large, international, randomized trials [3-6]. A multivalent vaccine to all types of HPV known to cause cervical cancer may be ideal; however, the costs of large international studies may be prohibitive. There is evidence that cross-neutralization occurs, thereby broadening the coverage of the HPV vaccines and increasing the benefits of vaccine to a larger range of infected people. However, this concept remains controversial. This editorial reviews the evidence for cross-protection in current clinical trials, in vitro work and epidemiologic studies.

Recently, Paavonen et al. published an interim analysis of a large, randomized, placebo-controlled trial of a HPV 16/18 L1 virus-like particle (VLP) vaccine [6]. While the primary end point was a decrease in high-grade cervical intraepithelial neoplasia (CIN 2+) associated with HPV 16 and 18, a decrease in persistent infections with other nonvaccine HPV types was also noted. This effect is termed ‘cross-protection.’ A decrease in persistent 6-month infections of 59.9 and 36.1% for HPV 45 and 31, respectively, was found, although this effect did not reach statistical significance against 12-month infections. Cross-protection of 31.6 and 46.5% was found for HPV 52 against 6- and 12-month infections, respectively.

Previous studies with longer follow-up (4.5 years) did not show protection against HPV 52, suggesting that this protective effect may diminish over time [4]. Broad protection was found against 12-month persistent infections, providing evidence that cross-neutralization may extend to HPV types other than 45 and 31 [6].

The HPV vaccine in this and other Phase III trials is composed of VLPs that resemble the HPV virion without being infectious. These VLPs are produced by expressing a given viral gene in an expression system, such as yeast or bacteria. In this case, the L1 capsid protein is used because it has been found to be highly immunogenic; for example, it is the component of the virus that the host recognizes and mounts an antibody response to. The sequence of the L1 open reading frame (ORF) determines the ‘type’ of the HPV. A new HPV type is designated when its DNA sequence differs by 10% or more from previously described types; differences between 2 and 10% are designated subtypes, and less than 2% differences are considered variants [7,8]. Over 100 different HPV types have been sequenced [7], and approximately 40 different types are known to infect the genital tract [1].

The first evidence that anti-HPV antibodies were capable of cross-neutralizing different HPV types was demonstrated with the low-risk HPV types 6 and 11. Types 6 and 11 are the most closely related of the HPV types, with capsid proteins sharing 92% homology, with only 39 of 501 amino acids being different. Despite being the most closely related of the HPV types, cross-neutralization was limited. Monoclonal antibodies raised against HPV 11 were able to neutralize HPV 11, but were unable to neutralize HPV 6 even at very high concentrations.

Conversely, polyclonal HPV 6 antisera was able to only weakly neutralize HPV 11. There were high titers of antibody reactive to HPV 11, but these did not appear to be targeting the neutralizing epitope [9]. It is not enough for an antibody to recognize and bind to a given antigen, rather it must recognize a site (epitope) on the antigen that will trigger neutralization. These epitopes appear to be highly type-specific, even in closely related HPV types.

Subsequent in vitro work by Giroglou et al. showed that cross-neutralization can occur in the high-risk types, but that it is specific for the type...
of antigen neutralized [10]. For example, HPV 18 pseudovirions were completely neutralized by HPV 45 antiserum. However, none of the antisera from other HPV types tested were effective against HPV 18. This was not surprising, since HPV 18 and 45 share a high degree of homology (88%), although, similar to the findings with HPV 6 and 11, it is not necessarily the case that antibodies to virus types with high homology are cross-protective. HPV 31 antibodies cross-neutralized HPV 33 more readily than HPV 16, although HPV 31 and 16 share a higher degree of homology (83.1%) than HPV 31 and 33 (78.4%) [10]. Overall relatedness with a high degree of homology (>85%) is important, but it appears that the location of certain amino acid residues may be the critical factor in defining the specificity of an antibody.

The L1 protein is highly conserved between virus types, but variable regions are interspersed throughout the conserved regions. With protein folding, the conserved regions are located on the inner, less exposed surfaces of the protein and the variable regions are on the outer portion of the capsid. Therefore, it is the variable regions on the outer surfaces that are more accessible to antibodies [11].

Developing a vaccine to recognize the conserved regions could maximize the number of HPV types neutralized by a given antibody. Combita et al. investigated cross-neutralization between HPV 16, 18, 31, 33, 45, 58 and 59, and identified antibodies that recognized these conserved regions, suggesting that cross-protection could occur. The activity of cross-neutralizing antibody was weak and represented 1% of homologous antibody-neutralizing activity. Thus, the authors questioned whether this low level of activity would render sufficient clinical protection in vivo [12].

Cross-neutralizing antibodies are not only limited in specificity, but are also less efficient. While in vitro studies demonstrated that HPV 31 and 45 VLPs were able to generate HPV 33- and HPV 18-neutralizing antibodies, respectively, the cross-neutralization was less efficient than neutralization mediated by the cognate antibody [10].

Additional in vitro evidence supporting cross-neutralization has recently been reported by Smith et al. [13]. They showed that the sera of women vaccinated with the quadrivalent HPV vaccine consisting of 6, 11, 16 and 18 VLPs was able to induce antibodies that neutralized HPV 45 in vitro.

This is evidence that the quadrivalent vaccine has cross-neutralization potential. Whether this phenomenon becomes apparent clinically may be revealed by future data from the vaccine trial.

Epidemiologic studies have not shown consistent evidence supporting the theory of cross-neutralization. While Ho et al. found that women who had persistently high levels of antibodies to HPV 16 VLPs had an approximately 50% reduced risk of non-HPV 16-related types [14], in a cohort study of 518 unvaccinated women, Thomas et al. were unable to find evidence for cross-protection [15]. They performed serial HPV DNA tests every 4 months, expecting to find decreased acquisition of HPV 31 after 16, HPV 18 after 45, HPV 6 after 11, and vice versa. They were unable to show that a prior infection with a phylogenetically related or unrelated type was associated with a decreased risk of acquiring a new HPV type. They noted that their study was small and likely underpowered to ascertain subtle relationships [15]. It is possible that the level of antibody response produced by a natural infection is not high enough to confer cross-protection, and therefore will not be detectable in an epidemiologic study of nonvaccinated individuals.

The antibody titers after vaccination are several logs higher than those achieved from natural exposure [5,6,16]. Perhaps the protection afforded by the HPV 16/18 vaccine against nonvaccine types that is not seen in epidemiologic studies may be due to a ‘supra-antibody’ effect, that is, supra-physiologic levels of antibody.

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Indeed, in vitro studies by Giroglou demonstrate that HPV 18 pseudovirions were completely neutralized by HPV 45 antiserum at -2log10 dilution and only 50% neutralized at -3log10 dilution [10]. Thus, cross-neutralization may occur in a dose-dependent fashion. When antibody levels are highest, at 7 months post-vaccination [6], one would expect the maximal cross-neutralization to occur. As antibody levels wane after 12 months [4], so may cross-neutralization. Hence, infections acquired during periods of lower antibody levels may not succumb to cross-neutralization. Similarly, infections acquired prior to the completion of the vaccination schedule may persist. This may explain why there was no statistically significant reduction of 12-month persistent infections with HPV types 31 and 45. It
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is likely that these 12-month persistent infections represent infections that were acquired early in the course of vaccinations, as the mean follow-up in this study was only 14.8 months [6].

Whether cross-protection for persistent infections is maintained over 5–10 years remains to be seen, and whether cross-neutralization is clinically significant in preventing dysplasia has not been established. Follow-up data from the ongoing large, randomized vaccine studies may be able to shed light on these issues.

The holy grail of HPV vaccine development is to create a vaccine that is not limited by type specificity, but instead is able to recognize and neutralize the entire spectrum of HPV types. While vaccine development has focused on the L1 capsid protein rather than its smaller counterpart, L2, recent research efforts into the latter are promising.

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Cross-neutralization of HPV 6, 16 and 52 was possible using antisera from sheep injected with HPV L2 capsid protein [7]. In addition, a particular HPV 16 L2 peptide has been identified and is believed to be a common neutralizing epitope [17]. Recently, Kondo et al. identified a segment of the HPV 16 L2 capsid protein that is on an exposed surface of the HPV virus. They injected this segment of L2 capsid into rabbits and found that the rabbit antisera was able to neutralize HPV 16, 18, 31 and 58 in vitro [18]. Another group demonstrated broad cross-neutralization against all oncogenic subtypes tested (HPV 16, 18, 31, 45, 52 and 58) in an animal model after vaccination with the HPV 16 N-terminal L2 polypeptide [19]. They have further identified a broadly cross-neutralizing epitope of HPV 16 L2 (peptides 17–36). Antiserum from rabbits injected with this L2 protein segment was able to neutralize pseudovirus HPV types 5, 6, 16, 18, 31, 45, 52 and 58 [20]. This work paves the way for the development of broad-coverage second-generation HPV vaccines.

Even if cross-protection is shown to be a reality, because of the prevalence of HPV types in current vaccines, 20–30% of cervical cancers will not be prevented. Vaccinated women will still be vulnerable to cervical cancer and will require continued surveillance. The women most in need of a vaccine against HPV are the women throughout the world who lack access to cervical cancer screening and basic healthcare. The technology to eradicate cervical cancer worldwide is within our grasp; we should continue our efforts to develop a broad-coverage vaccine.

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