issue. The bivalent vaccine was officially approved for use in Japan by the end of 2009, but there has been an ongoing debate on whether the use of HPV vaccine should be underpinned by tax-payers' money and if so, how much the government should spend.[9]

Despite its proven cost-effectiveness in other settings[10–12] a simple extrapolation of the costs and effectiveness of HPV vaccine in countries other than the Japanese setting is not appropriate because of the differences in cervical cancer epidemiology and health systems. The prevalence of HPV types differs between geographic regions. In the case of squamous cell carcinoma, HPV16 was the predominant type (46–63%) followed by HPV18 (10–14%), 45 (2–8%), 31 (2–7%) and 33 (3–5%) in all regions except Asia, where HPV types 58 (6%) and 52 (4%) were more frequently identified.[13] In Japan, HPV52 and HPV58 are most frequently found in squamous intraepithelial lesion following HPV16.[14] A relatively lower prevalence of HPV16 and HPV18 in Japan has cast doubt on the effectiveness of the current HPV vaccine when compared with other countries.[15]

Only one study has evaluated the cost-effectiveness of HPV vaccination in the Japanese setting.[16] However, the study did not compare strategies with a variable screening rate. Nor did it consider the effect of HPV type prevalence by age in Japan. Therefore, a cost-effectiveness analysis of screening coverage and vaccination, taking into account the age-specific prevalence by HPV type in the Japanese setting is urgently needed to inform and support policy decisions. Healthcare resources are limited; resources dedicated to screening and vaccination are no longer available for alternative healthcare uses and therefore the chosen strategy should represent a cost-effective use of scarce resources. The major objective of the present study is to assess the cost-effectiveness of universal vaccination against HPV in Japan from a societal perspective where the coverage of Pap smears is low and HPV oncogenic types are different from in other settings.

Methods

Natural history model of HPV infection

We developed a state-transition Markov model that simulates the natural history of HPV infection and carcinogenesis, in which transitions take place from one state to another at 1-month intervals (Figure 1). The model has 25 Markov states. The entry point into the model is girls aged 11 years with no previous exposure to HPV. We assumed that when girls/women enter the model, they start sexual activities, so acquiring a risk for HPV with the currently observed probabilities. In each cycle, they proceed to one of the four states: HPV16 and 18 DNA-positive group (HPV16 and 18), the other high-risk HPV DNA-positive group (other HR), the low-risk HPV DNA-positive group (LR), and the non-infected group (Normal) using monthly transition probabilities based on the systematic review of published literature.[17]

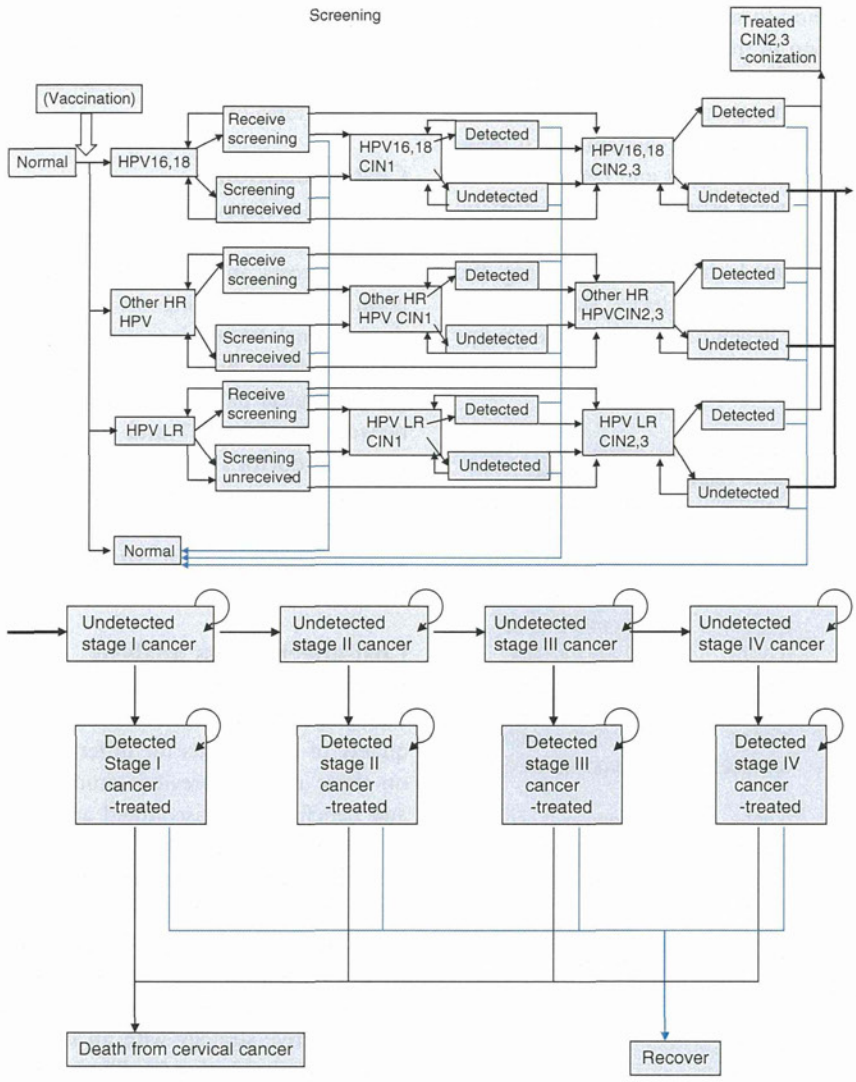
Each group follows a natural history unless they are screened. When cervical intraepithelial neoplasia (CIN) 2, 3 or invasive cancer is identified by the screening, a treatment intervention conditional on cancer stage is implemented. After the treatment for an invasive cancer, a certain proportion of patients die whereas others survive according to the survival probabilities compiled from the cancer registry data.[18,19] Age-dependent transition rates of disease progression from the susceptible to those infected with either HPV16/18, other HR (excluding HPV16 and 18) or LR were estimated from recent Japanese data.[20] All individuals are followed up for 50 years until they reach 60 years of age, which is the average retirement age in Japan, unless they die earlier.

Vaccine efficacy

The vaccine efficacy was evaluated in eight randomised controlled trials.[21,22] There was a substantial variation in follow-up periods and outcome measures among the studies. The World Health Organization adopted CIN2/3 as surrogate endpoints for cervical cancer in trials assessing vaccine efficacy.[21] In our model we used the relative risk of HPV16 and 18 persistent infection risk, as the vaccine immunises against the contraction of HPV. The hypothesis that persistent infection with one of the 15 carcinogenic HPV types is the fundamental cause of cervical cancer is clearly supported by scientific evidence.[23] We assumed relative risks of 0.12 (95% CI 0.03–0.48) for persistent HPV16 and 18 infection and 0.5 (range 0.3–0.7) for persistent HPV high-risk type excluding 16, 18.[21,22,24,25] Additionally, we assumed 100% lifetime protection against HPV16 and 18 once fully vaccinated.

Intervention strategies

The bivalent vaccine was approved for use in women and girls who are over 10 years old. The Japan Society of Obstetrics and Gynaecology recommended administering HPV vaccine among girls from 11 to 14 years of age as a priority, partly because they are old enough to understand the meaning of the vaccination and partly because the vaccination in this age group is efficient and ensures early protection against HPV with high immunogenicity. Therefore, for strategies which include vaccination, all 11-year-old girls are vaccinated at the entry point into the model. We assumed that there is no exposure to any HPV types before the entry to the model. At 20 years of age, they start receiving screening every 2 years according to the current Japanese recommendations.[5] Our reference strategy is the screening programme only with the current level of



*The probabilities to die from other causes are included at all each states.

Figure 1. HPV natural history model.

Table 1. Strategies

1. 20% coverage rate of screening with no vaccination
2. 50% coverage rate of screening with no vaccination
3. 80% coverage rate of screening with no vaccination
4. 20% coverage rate of screening with vaccination for all 11-year-old girls
5. 50% coverage rate of screening with vaccination for all 11-year-old girls
6. 80% coverage rate of screening with vaccination for all 11-year-old girls

coverage (i.e. 20%).[6,7] Table 1 summarises six strategies that were analysed in the present study. The sensitivity of the Pap smear was assumed to be 94.7% as previously reported.[26] The specificity (reported to be 98.9%) is not included in the model because screening will be repeated in false positives as determined by the cytology results.

Survival rates of women with cervical cancer

We used the data from life tables of Japanese vital registration to estimate the population-based mortality rates by age from cervical cancer and other competing risks.[27] Cumulative nationwide survival rates by cancer stages of FIGO classification were not available in Japan. We adopted the data from the US SEER programme (Surveillance Epidemiology and End Results),[18,19] which were calibrated using data from an existing Japanese regional cancer registry.[28]

Transition probabilities

Several natural history models of HPV have been developed and used in policy evaluations.[29,30] Whereas a particular

parameter has been common to several natural history models, there is a huge variation in the structure and parameters used in the previous models.[29] We used age-dependent type-specific HPV prevalence data from Japanese women[20] to derive transition probabilities from the susceptible to those infected with HPV16/18, other HR types and LR types.

Other model parameters were estimated from systematic literature reviews and then calibrated to the Japanese setting (Table 2).[17] We simulated the model by using the transition rates of CIN2, 3 to the undetected stage I cancer of HPV16/18 and other HR depending on their age groups in Japan. Then we adjusted them by using the data of age-dependent incident rates of cervical cancer. We validated the model by goodness-of-fit statistics using age-dependent mortality rates of cervical cancer.

Table 2. Transition rates

Variable	Baseline values	Range
Progression		
HPV DNA to CIN1		
Low-risk HPV	0.0264	0.0245–0.0284
High-risk 16, 18 HPV	0.0150	0.0026–0.0274
High-risk other HPV	0.0376	0.0271–0.0480
HPV DNA to CIN2, 3		
Low-risk HPV	0.00003	0.000003–0.00006
High-risk 16, 18 HPV	0.0012	0.000014–0.0024
High-risk other HPV	0.000025	0.000002–0.00005
CIN1 to CIN2, 3		
Low risk HPV	0.0003	0.00002–0.0005
High-risk 16, 18 HPV	0.0042	0.0001–0.0082
High-risk other HPV	0.0015	0.0001–0.0028
CIN2, 3 to undetected stage 1 cancer		
High-risk 16, 18 HPV	0.0049*	0.00001–0.0098
High-risk other HPV	0.0088*	0.00004–0.0176
Progression rates in unscreened women with cancer		
Stage I to stage II	0.0188	
Stage II to stage III	0.0250	
Stage III to stage IV	0.0375	
Regression		
HPV DNA to Normal		
Low-risk HPV	0.1951	
High-risk 16, 18 HPV	0.1951	
High-risk other HPV	0.1951	
CIN1 to Normal		
Low-risk HPV	0.0854	
High-risk 16, 18 HPV	0.1406	0.1316–0.1497
High-risk other HPV	0.0430	
CIN2, 3 to Normal (70% of women)		
Low-risk HPV	0.0145	0.0052–0.0238
High-risk 16, 18 HPV	0.0045	0.0010–0.0080
High-risk other HPV	0.0082	0.0029–0.0134
CIN2, 3 to HPV DNA or to CIN1 (15% of women each)		
Low-risk HPV	0.0031	0.0011–0.0051
High-risk 16, 18 HPV	0.0010	0.0002–0.0017
High-risk other HPV	0.0018	0.0006–0.0029

*Multiplied by age-dependent rate derived from calibration.

Cost estimation

A societal perspective was adopted for this cost analysis. Cost estimates are presented in Table 3 that include programme costs and time costs. We approximated the programme costs by using the current national tariff used by the national health insurance scheme.[31] These data were cross-validated by the cost of treatments and care for gynaecological patients at the University of Tokyo Hospital between August 2007 and November 2009. Both variable costs and doctor's fees are included in the programme costs according to the fee schedule set by the national tariff.[31] We estimated patients' time cost by using the national average hourly wage of part-time workers from a national survey.[32]

Cost-effectiveness analysis

We calculated quality-adjusted life-years (QALYs) from the model outputs on incidence, duration and mortality. The quality-of-life weights for different health states were based on those used in previous studies (Table 4).[33–35] All costs and benefits were discounted at 3%, a frequently used rate for cost-effective analysis done in Japanese settings.[36]

In line with a standard health economic evaluation, strategies are ranked in order of effectiveness after excluding dominated strategies.[37] Incremental cost-effectiveness ratios (ICERs) are then calculated for each strategy relative to the next best alternative. The preferred strategy is the most effective strategy with an ICER within the willingness to pay threshold of 4.5 million yen. A commonly applied threshold for acceptable cost-effectiveness in the USA is $50,000;[38] it is often used as a basis of cost-effective analysis in a Japanese setting.

Table 3. Cost data

Costs involving patient's time costs	Yen
Screening visit (Pap-test) per event	7460
CIN1 detected patient per month	4228
CIN2, 3 detection per event	28,360
Conisation cost per case	310,900
Treatment cost for stage I cancer case	664,300
Treatment cost for stage II cancer per case	2,869,900
Treatment cost for stage III cancer per case	3,066,500
Treatment cost for stage IV cancer per case	2,940,200
Average monthly wage for a Japanese case	226,100
Vaccination cost (for three doses/visits)	58,000

Table 4. Quality of life weights

Variable	Baseline values	Range
Quality of life weights for CIN		
CIN1	0.97	0.97–1.00
CIN2, 3	0.93	0.93–1.00
Quality of life weights for invasive cancer		
Stage I	0.65	0.49–0.81
Stage II	0.56	0.42–0.70
Stage III	0.56	0.42–0.70
Stage IV	0.48	0.36–0.60
Quality of life weights after treatment for invasive cancer		
Stage I	0.97	0.73–0.99
Stage II	0.9	0.68–0.98
Stage III	0.9	0.68–0.98
Stage IV	0.62	0.47–0.78

Results

Reduction in lifetime risk of cancer

Figure 2 shows the lifetime risk of cervical cancer by strategy estimated from a two-dimensional probabilistic sensitivity analysis. The range represents the minimum and maximum numbers of cervical cancer incidence per 100 000 population and its interquartile range (IQR). The bars represent the median value. Increasing the coverage of screening from the current level of 20–50 and 80% will substantially reduce the number of incident cervical cancer cases by 45.5% (IQR 42.0–48.7) and 63.1% (IQR 60.5–65.7), respectively. Combined strategies of 20, 50 and 80% screening coverage rate yields, respectively, a 66.1% (IQR 68.3–64.2), 80.9% (IQR 78.6–83.3) and 86.8% (IQR 85.4–87.9) reduction in cervical cancer incidence.

Total costs and QALYs of vaccination and screening programmes

Total QALYs gained per 100 000 population for each strategy showed slight increase as the screening coverage increases and the universal vaccination is added (Figure 3). Figure 4 shows cost per person for each strategy. The squares represent average values and the range represents average value ± 2 SD. Costs of strategies including vaccination are approximately four times higher than that of strategies without vaccination. Increasing the screening coverage rate was cheaper than introducing vaccination for all 11-year-old girls.

Incremental cost-effectiveness ratio

Table 5 shows the ICER of each strategy compared with its next best alternative strategy. Using the default model values, 50% screening coverage with a vaccination strategy was the most cost-effective when using a willingness to pay for a QALY threshold of 4,500,000 yen (≅ US$500,000) (Figure 5).

Sensitivity analysis on vaccine efficacy

We performed a sensitivity analysis on vaccine efficacy. The vaccine efficacy is determined by the combination of risk ratios to acquire HPV16/18 and other HR in our model. Table 6 shows cost and QALYs derived from the reference vaccine efficacy, minimum and maximum vaccine efficacy per 1000 people. Differences in vaccine efficacy would result in the differences in programme costs ranging from approximately 4,000,000–8,000,000 yen (≅US$480,000–960,000).

Table 7 shows the ICERs derived from the sensitivity analysis. The current strategy is dominated by strategies with a higher screening rate. A screening rate of 20% with a vaccination strategy is always ruled out because of extended dominance. The ICER for a screening rate of 50 and 80% with vaccination strategies was sensitive to the

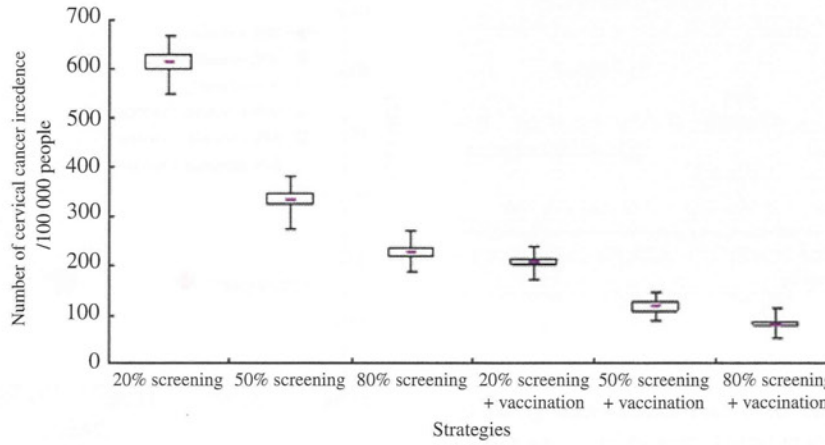


Figure 2. Lifetime risk of cancer for each strategy.

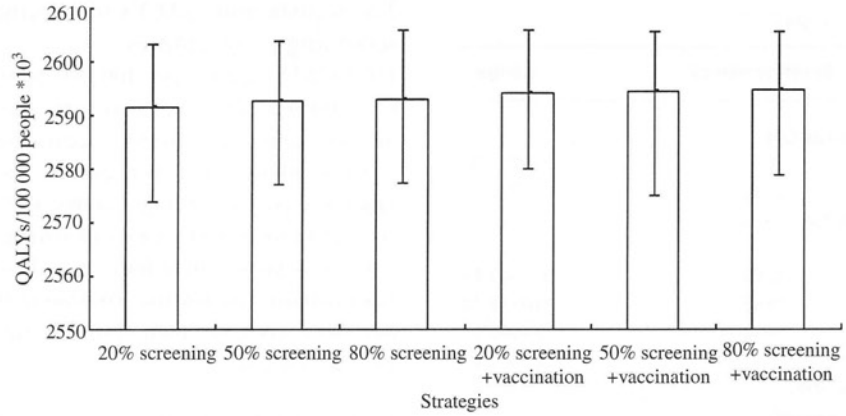


Figure 3. Total QALYs per 100,000 people for each strategy.

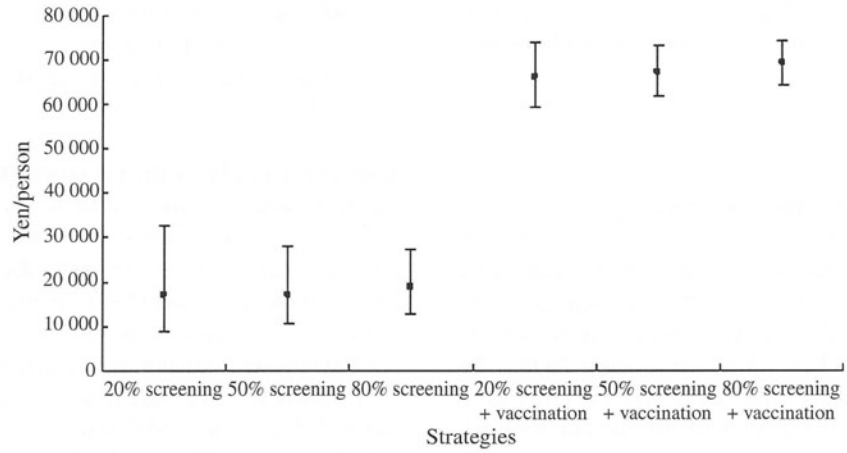


Figure 4. Cost per person for each strategy.

Table 5. Cost effectiveness of alternative screening and vaccination strategies

Strategy	Incremental cost effectiveness Ratio* (Yen/QALY)	
20% Screening	–	Dominated
50% Screening	658	
80% Screening	571 015	
20% Screening + vaccination	–	Extended Dominance
50% Screening + vaccination	2 920 636	
80% Screening + vaccination	8 568 182	not cost effective

*Ratio of additional costs and benefits of a particular strategy compared with the previous strategy.

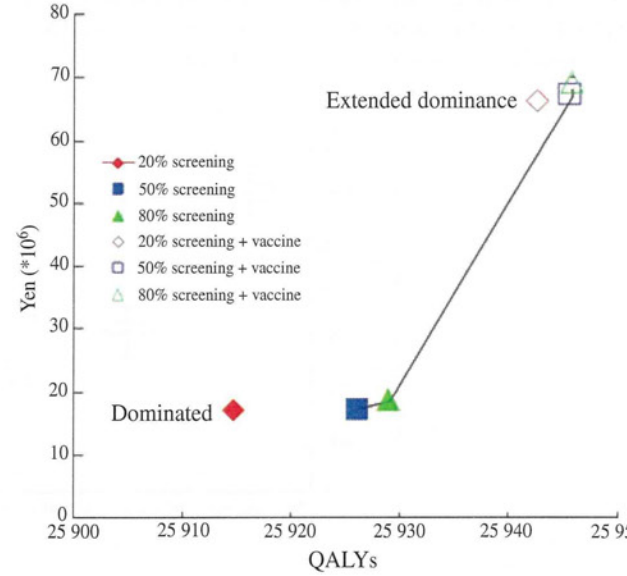


Figure 5. Cost and QALYs per 1000 people.

differences in incremental costs and effectiveness given by the result of a two-dimensional probabilistic sensitivity analysis of the model with each vaccine efficacy. With the

Table 6. Costs and QALYs per 1000 people of varied vaccine effect

Strategy	Minimum vaccine effect*		Baseline vaccine effect*		Maximum vaccine effect*	
	Cost (¥)	QALYs	Cost (¥)	QALYs	Cost (¥)	QALYs
Screening 20% + vaccination	69,561,000	25 933.88	66,114,000	25 942.64	62,628,000	25 950.77
Screening 50% + vaccination	70,300,000	25 937.33	67,334,000	25 945.54	64,300,000	25 953.22
Screening 80% + vaccination	72,129,000	25 940.81	69,219,000	25 945.76	66,277,000	25 953.07

*Minimum vaccine effect means relative risks of 0.48 for persistent HPV16 and 18 infection and 0.7 for persistent HPV high-risk type excluding 16, 18 infection. Baseline vaccine effect means relative risks of 0.12 for persistent HPV16 and 18 infection and 0.5 for persistent HPV high-risk type excluding 16, 18 infection. Maximum vaccine effect means relative risks of 0.03 for persistent HPV16 and 18 infection and 0.3 for persistent HPV high-risk type excluding 16, 18 infection.

Table 7. Sensitivity analysis on vaccine effect (ICER)*

Strategy	Minimum vaccine effect**	Baseline vaccine effect**	Maximum vaccine effect**
Screening 20%	Dominated	Dominated	Dominated
Screening 50%	658	658	658
Screening 80%	Extended dominance	571 015	571 015
Screening 20% + vaccination	Extended dominance	Extended dominance	Extended dominance
Screening 50% + vaccination	Extended dominance	2 920 636	1 874 867
Screening 80% + vaccination	3 745 442	8 568 182	Dominated

*Incremental cost effectiveness ratio (Yen/QALY).
**Minimum vaccine effect means relative risks of 0.48 for persistent HPV16 and 18 infection and 0.7 for persistent HPV high-risk type excluding 16, 18 infection. Baseline vaccine effect means relative risks of 0.12 for persistent HPV16 and 18 infection and 0.5 for persistent HPV high-risk type excluding HPV16, 18 infection. Maximum vaccine effect means relative risks of 0.03 for persistent HPV16 and 18 infection and 0.3 for persistent HPV high-risk type excluding 16, 18 infection.

minimum efficacy, a combined strategy of 80% screening and universal vaccination is most cost-effective. On the other hand, with the maximum and baseline vaccine efficacy, a combined strategy of 50% screening and universal vaccination remains most cost-effective.

Discussion

The introduction of HPV vaccine to the Japanese population has been controversial because the coverage of Pap smear screening is low and the prevalence of HPV types is different from that observed in Western countries.

To date there has been only one study that has assessed the impact of introducing HPV vaccine in Japan.[16] However, this study suffered from several major limitations. It did not distinguish health status related to HPV type 16 and 18 from other high-risk types. We modelled the natural history of each HPV type status; HPV16/18, other HR, and LR. We used different vaccine efficacies depending on the HPV types with a range that was derived from a meta-analysis of the available evidence. The previous study also did not include strategies of varied screening rates without vaccination. The authors analysed the effect of screening atthe currently observed levels ranging from 13.6 to 24.7%, and so the impact of increasing Pap smear coverage was not considered. Instead, the present study compared the strategies of varied screening rates ranging from 20 to 80%.

Our analysis suggests that increasing cervical cancer screening coverage to 50% would halve the incidence of cervical cancer and save programme costs and that the introduction of HPV vaccination would reduce the incidence by two-thirds but result in a four-fold increase in programme costs. Using the model's default values, a combined strategy to expand the coverage of cancer screening up to 50% and the introduction of universal vaccination would be most cost-effective. The results are robust with sensitivity analysis in which the optimum coverage level most likely lies somewhere between 50 and 80%. Our result confirms the need for expanding coverage for Pap smears in Japan as previously suggested,[39] to maximise the impact of the cervical cancer strategy regardless of whether a national vaccine programme is also implemented.

The detection rate of HPV16 and 18 among women with cervical cancer in Japan is reported to be lower than that in other countries.[14] We used the latest age-dependent prevalence data, which consistently show that the younger population has a higher detection rate of HPV16 and 18 than the older population.[20] The prevention of cervical cancer in a young person shows larger QALYs gained than that of an older person because of the longer remaining life expectancy. Hence the effect of vaccine on cancer incidence or QALYs is not as low as might otherwise be expected.

The present study has several limitations. First, we assumed life-long lasting immunity acquired by the vaccine. The vaccine has only been recently introduced, and the latest evidence shows 7.3 years of efficacy and immunogenicity of the vaccine, which was derived from the population of the initial placebo-controlled study.[40] If additional vaccination is required to maintain immunity in the future, then programme costs are slightly underestimated. Second, there is no population-based survival data of women with cervical cancer by stages of FIGO. These data are essential when building a model. However, we managed to adopt and validate data from an existing Japanese regional cancer registry. Third, we did not incorporate the preferences of girls and their parents and the subsequent uptake of vaccine as a result of their preferences. Both effects and costs may be overestimated in that sense. Finally, we did not include the cost for campaigns to increase the coverage of screening and/or vaccination in this analysis, which may underestimate the programme costs but such a bias is minimal given the fact that the majority of costs is incurred by screening, vaccination and treatment interventions.

Vaccination for HPV is attracting considerable policy attention now as a strategy for cervical cancer prevention in Japan. Our analysis showed that increasing the rate of the current screening strategy would halve cancer incidence with a similar cost to the current screening strategy, though vaccination strategies may also be cost effective. We suggest further efforts to expand the current screening programme regardless of what support is provided for vaccination.

Some of the reasons why Pap smear coverage is so low in Japan relate to a lack of knowledge and from the fact that the financial support of the screening programme from the Ministry of Health, Labour and Welfare was discontinued because it was included in the general ones in 1998. Most cities, towns and villages decided to reduce the cost for the screening programme.[41–43] Free tickets for the Pap smear were provided under supplemental budgets for 2009. Distributing free tickets to a target population of certain ages showed a significant increase in the coverage rate by 2.8 times.[44] We need to continue endeavours to increase coverage by effective interventions such as providing free tickets and undertaking awareness campaigns. The involvement of gynaecologists in school education will also support the enhancement of knowledge about cervical cancer prevention and help to increase the coverage rate of screening as has been seen in other countries.[45,46]

Our analysis showed that introducing the HPV vaccination for all 11-year-old girls would reduce cervical cancer incidence to 33.9% with a net cost of only 49,000 yen per person (taking into account the social burden of cancer). Vaccinating all 11-year-old girls would cost 33.7 billion yen. Our analysis showed the cost-effectiveness of vaccination and that it would save future costs. It is important to give priority to policy which is evidence based medically and economically. If the prevalence of HPV infection is reduced as a result of universal vaccination, as our model predicts, then it may be possible to extend the interval between routine screens or to increase the age at which screening is first offered, as suggested in other cost-effectiveness studies.[34,47] The use of the HPV-DNA test in the screening programme is one choice that should be evaluated in the future.

In conclusion, the introduction of HPV vaccine in Japan is cost-effective as in other countries. It is more cost-effective to increase the coverage of the Pap smear along with the universal administration of HPV vaccine. Only by doing so, can the scarce healthcare resources be efficiently and effectively used to reduce the burden from cervical cancer in Japan.

Disclosure of interests

None of the authors have any conflicts of interest to declare.

Contribution to authorship

NY contributed to the study design of the current paper, model construction, data acquisition, data analysis and interpretation, drafting and revising the manuscript. RM contributed to the study design of the current paper, model construction, results interpretation and revising the manuscript. PJ contributed to the model construction, results interpretation and the critical review of the manuscript. YO contributed to the study design of the current paper. KK contributed to the model construction, data acquisition and interpretation of the results. KS and YT contributed to the study design of the current paper and interpretation of the results. All authors approved the final version of the manuscript.

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Characterization of Gut-Derived Intraepithelial Lymphocyte (IEL) Residing in Human Papillomavirus (HPV)-Infected Intraepithelial Neoplastic Lesions

Satoko Kojima[1], Kei Kawana[1], Tomoyuki Fujii[1], Terufumi Yokoyama[2], Shiho Miura[1], Kensuke Tomio[1], Ayako Tomio[1], Aki Yamashita[1], Katsuyuki Adachi[1], Hidetaka Sato[1], Takeshi Nagamatsu[1], Danny J. Schust[3], Shiro Kozuma[1], Yuji Taketani[1]

[1]Faculty of Medicine, Department of Obstetrics and Gynecology, University of Tokyo, Hongo, Bunkyo-ku, Tokyo, Japan;
[2]GENOLAC BL Corp. 503, Okinawa Industry Support Center, Oroku, Naha, Okinawa, Japan;
[3]Division of Reproductive Endocrinology and Fertility, Department of Obstetrics, Gynecology and Women's Health, University of Missouri School of Medicine, Columbia, MO, USA

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Correspondence
Kei Kawana, MD, PhD, Department of Obstetrics and Gynecology, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan.
E-mail: kkawana-tky@umin.ac.jp

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Problem
Mucosal T cells are the most likely direct effectors in host anti-human papillomavirus adaptive immunity and regression of cervical intraepithelial neoplasia (CIN) lesions. There are no studies addressing intraepithelial lymphocytes (IELs) in CIN lesions.

Method of study
Cervical lymphocytes were collected using cytobrushes from patients with CIN and analyzed by FACS analysis. Comparisons were made between populations of cervical T cells in CIN regressors and non-regressors.

Results
A median of 74% of cervical lymphocytes were $CD3^+$ T cells. Populations of integrin $\alpha E\beta 7^+$ IEL in CIN lesions varied markedly among patients (6–57%). Approximately half of integrin $\beta 7^+$ T cells were CD45RA-negative memory T cells. The number of integrin $\alpha E\beta 7^+$ cells among cervical T cells was significantly higher in CIN regressors when compared to non-regressors.

Conclusion
Higher cervical IEL numbers are associated with spontaneous regression of CIN. Accumulation of cervical integrin $\alpha E\beta 7^+$ IEL may be necessary for local adaptive effector functions.

Introduction

Lymphocytes involved in the mucosal immune system are found in the inductive sites of organized mucosa-associated lymphoid tissues (MALT) and in a variety of effector sites such as the mucosa of the intestine, respiratory tract, and genital tract.[1] The efficient homing of lymphocytes to the gut is dependent on the homing receptors integrin α4β7 and C-C chemokine receptor type 9 (CCR9). Lymphocyte-expressed integrin α4β7 and CCR9 bind to their natural ligands, mucosal addressin cell adhesion molecule-1 (MAdCAM-1) and CCL25 (TECK), respectively, which are expressed on the cell surface of

endothelial cells in submucosal post-capillary venules.[1] In the intestine, mucosal dendritic cells (DCs) in gut-associated lymphoid tissues (GALT) regulate the expression of integrin $\alpha4\beta7$ on activated effector and regulatory lymphocytes in a retinoic acid–dependent manner.[1–3] Mucosal T cells expressing integrin $\alpha4\beta7^+$ are known to circulate in peripheral blood from inductive sites and to home to the lamina propria (LP) at effector sites via $\alpha4\beta7$-MAdCAM-1 and CCR9-CCL25 interactions.[4] Integrin $\alpha4\beta7^+$ T cells can differentiate into $\alpha E\beta7^+$ T cells upon exposure to TGF-β,[5] and the expression of integrin $\alpha E\beta7$ facilitates the retention of lymphocytes in the epithelium via interactions with E-cadherin.[4] Integrin $\alpha E\beta7$ is a specific marker of intraepithelial lymphocytes (IELs) residing in mucosal epithelia, and those cells expressing this antigen on their surface were initially educated in the gut.

The cervical mucosa is a very common site for pathogen invasion and is the primary transmission site for human papillomavirus (HPV), *Chlamydia trachomatis*, and human immune deficiency virus type 1 (HIV-1). A well-organized mucosal defense system in the cervical mucosa is critical to human health. Mucosal epithelial cells in the human cervix are active participants in such immunological protection.[6] However, the lymphocytes populating the cervical mucosal tissues, especially cervical IELs, have been poorly studied. Mucosal T cells in the murine genital tract express a large amount of integrin $\alpha4\beta7$ on their cell surface,[7] and MAdCAM-1 is expressed on endothelial cells in the submucosa of murine fallopian tubes infected with *C. trachomatis*.[8] Several studies have demonstrated that human genital mucosa expresses MAdCAM-1 endogenously[9] and that GALT-derived integrin $\alpha4/E\beta7^+$ T cells home to the genital mucosa.[10,11] This T-cell homing and the expression of integrin αE increase in the presence of cervicitis and vaginitis.[10,11] Although integrin $\beta7^+$ mucosal T cells have been found in the cervical mucosa, a local inductive site (i.e., MALT) has never been demonstrated histologically.[11] We hypothesized that GALT may act as the inductive site for cervical IELs.

Human papillomavirus infection is a major cause of cervical cancer, and its precursor lesion, cervical intraepithelial neoplasia (CIN), develops in the epithelium. Natural history studies of CIN[12,13] show that most infections and CIN lesions resolve spontaneously but some persist and progress to cervical cancer. Studies showing that HIV-infected women and patients who are under treatment with immunosuppressive agents have an increased incidence of CIN lesions[14,15] suggest that cell-mediated immune response against HPV antigens is important in the control of HPV infection and progression to CIN. More controversial are the relative roles of systemic and local mucosal immune responses in the HPV pathogenesis. Trimble et al.[16] reported that naturally occurring systemic immune responses to HPV antigens do not predict regression of CIN 2/3 lesions, but Nakagawa et al.[17] demonstrated a positive association between systemic cell-mediated immune responses to HPV E6 and HPV/CIN regression.

We studied the local mucosal cell–mediated immune response to HPV antigens by characterizing cervical mucosal immune cells collected non-invasively, using only a cytobrush. We confirmed that the collected $CD3^+$ cervical T cells were intraepithelial in origin (integrin $\alpha E\beta7^+$ IELs). Approximately half of the integrin $\beta7^+$ T cells were memory T cells. Finally, integrin $\beta7^+$ intraepithelial T cells increased significantly in the patients whose CIN lesions regressed spontaneously regardless of HPV genotype.

Materials and methods

Study Population

Cervical cell samples were collected using a cytobrush from 86 patients under observation after being diagnosed with CIN by colposcopically directed biopsy. All women gave written informed consent, and the Research Ethics Committee of the University of Tokyo approved all aspects of the study. Patients with known, symptomatic, or macroscopically visible vaginal inflammation or sexually transmitted infections were excluded from our study. Samples for HPV genotyping were collected at the first follow-up examination after diagnosis. Cervical lymphocytes were collected from non-menstruating patients at their latest follow-up visit. To study the potential association between cervical IEL characteristics and CIN progression, CIN patients with the regression of cervical cytology (cases) were matched with control patients who did not exhibit cytologic regression over the same time period (measured from initial detection of abnormal cytology). In this study, cytological regression was defined as normal cytology at two or more consecutive evaluations conducted at 3 to 4-month intervals. Thirteen patients were enrolled in the regression group, and the median

follow-up duration was 27 (12–38) months. Thirteen pairs of follow-up time-matched patients with persistent cytological abnormalities were enrolled in the non-regression group, and the median follow-up time was 24 (12–40.5) months.

HPV Genotyping

DNA was extracted from cervical smear samples using the DNeasy Blood Mini Kit (Qiagen, Crawley, UK). HPV genotyping was performed using the PGMY-CHUV assay method.[18] Briefly, standard PCR was conducted using the PGMY09/11 L1 consensus primer set and human leukocyte antigen-DQ (HLA-DQ) primer sets. Reverse blotting hybridization was performed. Heat-denatured PCR amplicons were hybridized to specific probes for 32 HPV genotypes and HLA-DQ reference samples. The virological background (HPV genotyping) of 86 patients in our study was shown in Table I. Here, HPVs 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 68, 73, and 82 were defined as high-risk HPVs according to International Agency for Research on Cancer multicenter study.[19]

Table I Human Papillomavirus (HPV) Genotype Distribution

HPV type	Total numbers (%)
16	19 (18.4)
18	7 (6.8)
31	2 (1.9)
33	1 (1.0)
35	1 (1.0)
39	1 (1.0)
45	1 (1.0)
51	7 (6.8)
52	20 (19.4)
53	4 (3.9)
56	3 (2.9)
58	12 (11.7)
59	3 (2.9)
68	3 (2.9)
82	1 (1.0)
6	2 (1.9)
54	1 (1.0)
55	1 (1.0)
66	4 (3.9)
69	1 (1.0)
70	3 (2.9)
83	3 (2.9)
84	2 (1.9)
Total	103 (100)

Patients infected with multiple HPV types were included. Of 86 patients, 32 (37%) were infected with multiple types. HPVs 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 68, 73, and 82 were defined as high-risk HPVs.[19]

Collection and Processing of Cervical Specimens

Cervical cells were collected using a Digene cytobrush as described previously.[20] The cytobrush was inserted into the cervical os and rotated several times. The cytobrush was placed in a 15-mL tube containing R10 media [RPMI-1640 medium, supplemented with 10% fetal calf serum (FCS), 100 mg/mL streptomycin, and 2.5 μg/mL amphotericin B] and an anticoagulant (0.1 IU/mL of heparin and 8 nM EDTA). After incubating the sample with 5 mM DL-dithiothreitol at 37°C for 15 min with shaking, the cytobrush was removed. The tube was centrifuged at $330 \times g$ for 4 min. The pellet was resuspended in 10 mL of 40% Percoll, layered onto 70% Percoll, and centrifuged at $480 \times g$ for 18 min. The mononuclear cells at the Percoll interface were removed and washed with PBS. Cell viability was >95%, as confirmed by trypan blue exclusion test, and fresh samples were immediately used for further analysis.

Immunolabeling and Flow Cytometry

Cervical immune cell preparations were immunolabeled, incubated on ice for 30 min, washed twice with FACS buffer (10% FCS, 1 mM EDTA, and 10 mM NaN_3) and fixed by adding paraformaldehyde in PBS to a final concentration of 1%.

The following fluorochrome-conjugated mouse monoclonal antibodies specific for human leukocyte surface antigens were used: a fluorescein isothiocyanate (FITC)-conjugated pan leukocyte marker (FITC-anti CD45), a B lymphocyte marker (FITC-anti CD19), a cytotoxic T-cell marker (FITC-anti CD8), a helper T-cell marker (FITC-anti CD4), an integrin β7 marker (FITC-anti integrin β7), a phycoerythrin (PE)-conjugated integrin α4 marker (PE-anti integrin α4), an integrin αE marker (PE-anti integrin αE), a C-C chemokine receptor type 9 marker (PE-anti CCR9), a marker for naïve cells (PE-anti CD45RA), a phycoerythrin cyanine 5 (PC5)-conjugated pan T lymphocyte marker (PC5-anti CD3), a natural killer cell marker (PC5-anti CD56), and an allophycocyanin (APC)-conjugated pan T lymphocyte marker (APC-anti CD3). Cell preparations were labeled in parallel with appropriate isotype control

antibodies. Antibodies were purchased from eBioscience (San Diego, CA, USA) and Beckman Coulter (Brea, CA, USA). Data were acquired using three-color flow cytometry on FACSCalibar (Becton-Dickinson, Texarkana, TX, USA). The positions of lymphocytes and monocytes were determined on the forward scatter versus side scatter (SSC) profile. The positions of pan-lymphocytes and T lymphocytes were determined by CD45 and CD3 gating, respectively. As the percentage of B cells among cervical lymphocytes is known to be low (less than a few percentage) when compared to the 20% level seen in peripheral blood,[20] the presence of elevated $CD19^+$ B cells in cervical specimens would indicate contamination with peripheral blood. For our investigations, cervical samples with more than 3% B cells were excluded from analysis.

Statistical Analysis

Statistical analyses, including calculation of medians and interquartile ranges (IQRs), were performed using the commercial statistical software package JMP® (SAS, Cary, NC, USA). Wilcoxon rank sum test or Fisher's exact test was applied for matched paired comparisons. *P*-values ≤ 0.05 were considered significant.

Results

Purification of Cervical Leukocytes Collected from CIN Lesions

To characterize mucosal cellular immune responses in HPV-infected lesions, cervical samples, including exfoliated epithelial cells and cervical lymphocytes, were collected from CIN lesions positive for any HPV genotype using a cytobrush. Cervical samples were fractionated over a discontinuous Percoll density gradient to remove cervical epithelial cells, and the layer between Percoll and culture medium was collected. Cervical lymphocytes were identified among isolated cells using standard SSC and CD45 gating (Fig. 1). Approximately 10^4–10^5 $CD45^+$ cells were isolated from patients' cervices. $CD45^+$ cells primarily consisted of lymphocytes (Fig. 1, circle) and granulocytes (Fig. 1, square). A minority of the cells included in the square in Fig. 1 were monocytes (data not shown). Two representative cases are provided in Fig. 1: the upper panel represents a patient with numerous granulocytes and a relatively small population of $CD45^+$ lymphocytes (10%), whereas the lower panel represents a patient with few granulocytes and a high number of lymphocytes (30%).

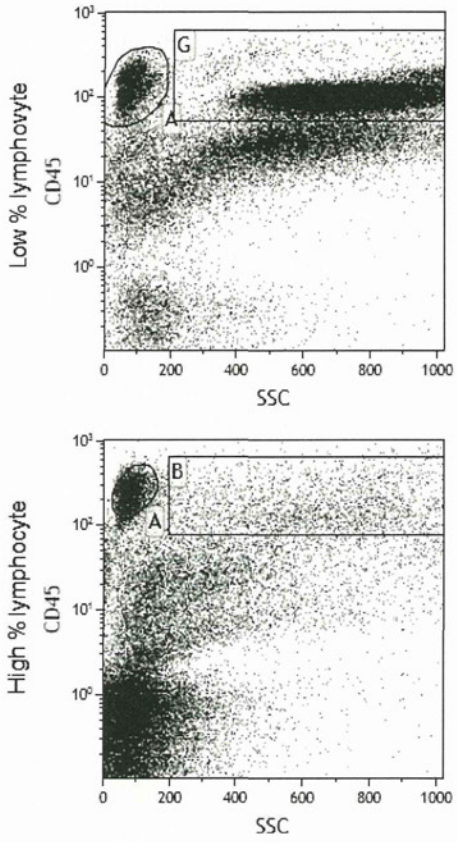

Fig. 1 Flow cytometric analysis of cervical mucosal cells using CD45/SSC gating. Processed cervical specimens were analyzed by flow cytometry and CD45/SSC gating. $CD45^+$ cervical leukocytes are comprised of lymphocytes (circle) and granulocytes/monocytes (square). Upper and lower panels were representative of patients with low (about 10%) and high (about 30%) numbers of lymphocytes among their $CD45^+$ cervical leukocytes, respectively. The absolute number of isolated cervical lymphocytes remained relatively constant among study subjects.

Characterization of Cervical T Cells in CIN Lesions

The majority of cervical lymphocytes isolated from CIN lesions were $CD3^+$ T cells [median 74% (IQR: 59–82)]. $CD19^+$ B cells were rarely found [median 0.45% (IQR: 0.04–1.40)]. In Fig. 2, CD3-gated cervical T cells were characterized by flow cytometry, and each median, IQR, and maximum/minimum range is indicated using horizontal lines, boxes, and vertical length lines, respectively. A median of 54%

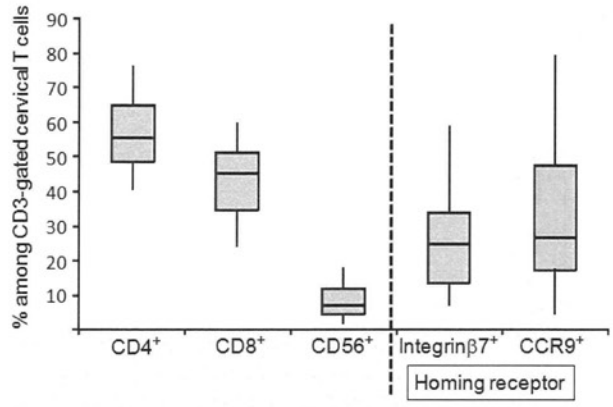

Fig. 2 Characterization of the cervical $CD3^+$ lymphocytes. CD3-gated cervical T cells consisted of $CD3^+$ $CD4^+$ T cells [median 54% (IQR: 49–65), $n = 28$], $CD3^+$ $CD8^+$ T cells [median 46% (IQR: 35–51), $n = 28$], and $CD3^+$ $CD56^+$ natural killer T cells [median 5.6% (IQR: 4.5–12), $n = 17$]. Twenty-four percentage (IQR: 13–34, $n = 43$) and 27% (IQR: 17–47, $n = 27$) of cervical T cells were integrin $\beta7^+$ and $CCR9^+$, respectively. Each median, IQR, and maximum/minimum range is indicated using horizontal lines, boxes, and vertical length lines, respectively.

(IQR: 49–65) of cervical T cells were $CD3^+$ and $CD4^+$, while a median of 46% (IQR: 35–51) expressed CD3 and CD8, demonstrating that $CD8^+$ T cells are relatively abundant among cervical T cells. The CD4/CD8 ratio of 1.15 in the cervix was clearly lower than the value of 2.0 found in peripheral blood. Among $CD3^+$ cells, a median of 5.6% (IQR: 4.5–12) were $CD56^+$ natural killer T (NKT) cells.

Those cervical $CD3^+$ T cells that were originally derived in the gut were defined by expression of the gut mucosa–specific cell-surface antigens integrin $\beta7^+$ and $CCR9^+$. A median of 24% (IQR: 13–34) of cervical T cells expressed integrin β7 and 27% (IQR: 17–47) expressed CCR9 (Fig. 2).

Notably, more than 90% (median: 99.1, IQR: 95.3–100) of the integrin $\beta7^+$ cells co-expressed the αE subunit (integrin $\alpha E\beta7^+$ cells; Fig. 3a). Integrin $\alpha4^+$ cells were rarely present among the integrin $\beta7^+$ cells (Fig. 3b). Approximately 40% (median: 40.1, IQR: 33.2–44.2) of cervical integrin $\beta7^+$ cells were integrin $\alpha E\beta7^+$ $CCR9^+$ double positive (Fig. 3c).

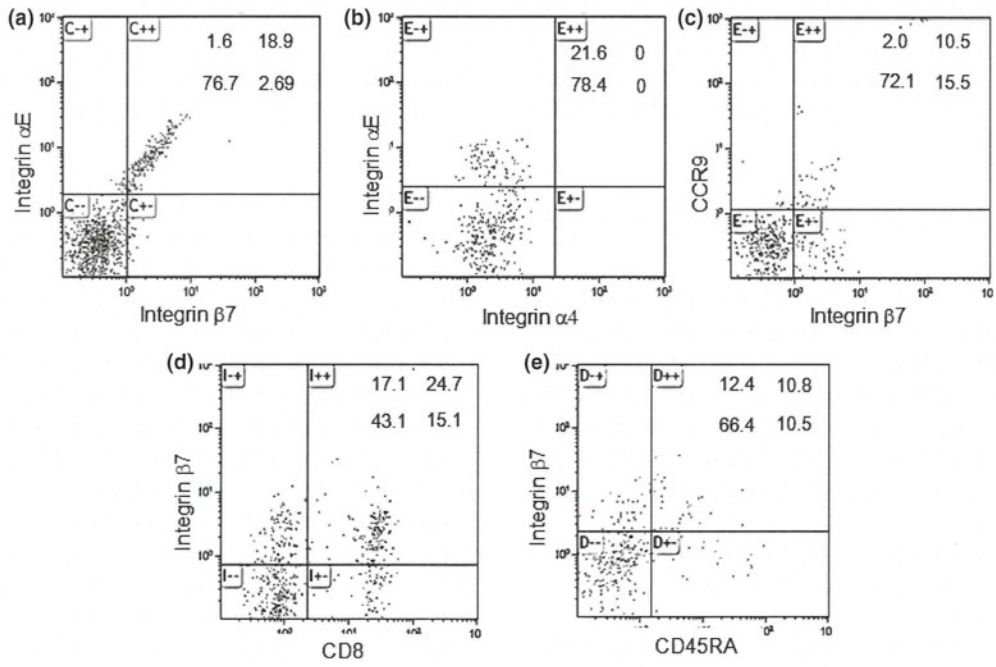

Fig. 3 Characterization of CD8, CD45RA, and homing receptors specific for gut-derived mucosal T cells among $CD3^+$ cervical T cells. Representative flow cytometry analyses of CD3-gated cervical T cells. (a) More than 90% of integrin $\beta7^+$ cervical T cells were integrin αE^+ intraepithelial lymphocyte. (b) Integrin $\alpha4^+$ LPL were negligible in our cervical samples. (c) Among integrin $\beta7^+$ cells, approximately 40% were $CCR9^+$. (d) Forty-two percentage of total cervical T cells and 53% of integrin $\beta7^+$ T cells were $CD8^+$. (e) About half of the integrin $\beta7^+$ T cells were CD45RA-negative memory cells.

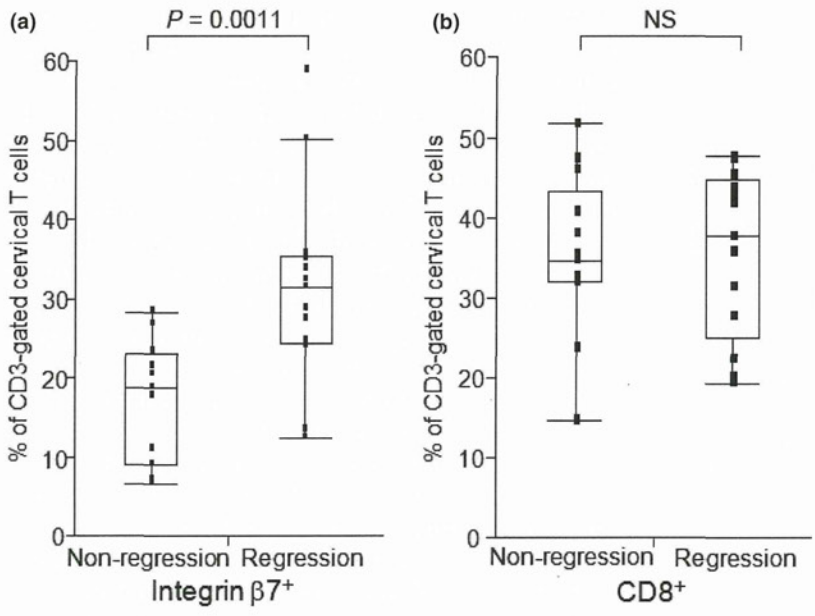

Fig. 4 Association between gut-derived cervical intraepithelial lymphocyte and cervical intraepithelial neoplasia (CIN) regression. Populations of integrin $\beta7^+$ (a) and $CD8^+$ (b) cells among $CD3^+$ cervical T cells were compared between CIN regression (n = 13) and non-regression (n = 13) groups, paired according to follow-up duration. A P-value ≤0.05 was considered significant using Wilcoxon rank test comparisons.

$CD8^+$ memory T cells are essential for adaptive cytotoxic immune responses to CIN.[21,22] Among our patients with CIN, the proportion of integrin $\beta7^+$ cervical T cells that expressed CD8 [median 53% (IQR: 28–69)] was greater than that for total cervical T cells (Fig. 3d). Approximately half [median 43% (IQR: 31–57)] of integrin $\beta7^+$ T cells were CD45RO memory T cells, while the other half were CD45RA effector T cells (Fig. 3e).

Association of Gut-derived Cervical IEL with CIN Course

Integrin β7 is a more ubiquitous homing receptor in mucosal lymphocytes rather than integrin αE or α4. To determine whether there was an association between the presence of gut-derived cervical IEL and spontaneous regression of CIN, comparisons were made between populations of integrin $\beta7^+$ $CD3^+$ and $CD8^+$ $CD3^+$ cervical T cells in CIN regressors (n = 13) and non-regressors (n = 13), paired according to their duration of follow-up. No significant differences were seen in the detection rates of high-risk HPV (69 versus 77%, P = 0.50), the squamous intraepithelial lesion (SIL) grade (high-grade SIL: 54 versus 54%, P = 0.65), and the median ages (34 years old versus 35) of patients in the regression and non-regression groups. The percentage of integrin $\beta7^+$ cervical T cells varied from 6 to 57% among the 26 study subjects. Among regressors, integrin $\beta7^+$ cervical T cells comprised a median of 31.6% (IQR: 24.5–35.5) of $CD3^+$ cervical T cells; the rate among non-regressors was 18.8% (IQR: 9.2–23.3), P = 0.0011 (Fig. 4a). In contrast, there was no difference in populations of $CD8^+$ $CD3^+$ cervical T cells between CIN regressors and non-regressors (Fig. 4b). The proportion of $CCR9^+$ and $CD45RA^+$ $CD3^+$ cervical T cells was likewise similar in the two groups (data not shown).

Discussion

Human papillomavirus preferentially infects, and CIN develops in the human cervical epithelium. It is clear that HPV antigens are recognized by the systemic cell-mediated immune system, but remains unclear whether systemic cellular immune responses predict the regression of CIN.[16,17] Local mucosal immune responses in the cervix are likely to be important in immunological clearance of CIN lesions. Integrin $\alpha4\beta7$ is essential for recruiting activated mucosal lymphocytes from the circulation into local LP in a manner entirely dependent on interaction between lymphocyte integrin $\alpha4\beta7$ and the MAdCAM-1 that is constitutively expressed on LP post-capillary venules.[23] In contrast, integrin αE (CD103) β7 is expressed by only 2% of circulating blood lymphocytes, but more than 90% of IEL and a minority of lamina propria lymphocyte (LPL); its ligand is E-cadherin expressed on the epithelial cells.[24] Activated integrin $\alpha4\beta7^+$ T cells differentiate within the

LP into integrin $\alpha E\beta 7^+$ T cells upon exposure to TGF-β locally secreted by epithelial cells.[5] Binding of integrin $\alpha E\beta 7$ to E-cadherin provokes retention of the activated IEL within the epithelium. Recognition of target epithelial cells by IELs is important in the initiation of cytolytic effector function by activated IELs and modulation of adaptive immune responses to control potentially destructive epithelial immunity. Adhesion of integrin $\alpha E\beta 7^+$ IEL to epithelial E-cadherin is promoted by CCL25–CCR9 interactions.[4] This suggests that, when compared to integrin $\alpha 4\beta 7^+$ LPL, integrin $\alpha E\beta 7^+$ IELs may be more directly linked to essential adaptive immune responses to target epithelial cells at local effector sites.

Several studies have reported that integrin $\alpha 4\beta 7$ is expressed on gut-derived mucosal lymphocytes within the cervix.[9,11] However, our data indicate that more than 90% of integrin $\beta 7^+$ T cells were positive for integrin αE and few express α4. Pudney et al.[10] have shown using immunohistochemistry that integrin $\alpha E\beta 7^+$ lymphocytes are primarily located in the epithelium of the ectocervix and often occur as focal accumulations in the LP of the transformation zone. Our brushing methodology enables us to preferentially collect cervical mucosal lymphocytes from the epithelium and occasionally from the LP. Others who have recently reported that nearly all cervical tissue T cells are integrin $\alpha 4\beta 7^{+9}$ used cervical tissue specimens and equally valuable methodologies that would be expected to isolate cells from deeper within the cervical tissue, possibly favoring isolation of LPL over cells tightly adhered to the epithelium.

Our cervical samples were contaminated by numerous granulocytes, a finding supported by several previous studies using cervical mucosa unlike peripheral blood samples.[10,11] Granulocyte contamination variability was likely the result of differing levels of cervical inflammation among patients. Although the number of lymphocytes among $CD45^+$ cervical leukocytes varied from 10 to 30%, the absolute number of cervical lymphocytes present in a sample appeared to be relatively constant and independent of patient source. The efficient homing of lymphocytes to the gut is dependent on the homing receptors integrin β7 and CCR9. We showed that integrin β7 and CCR9 did not always co-express. This agrees with reports showing that expression of the mucosal homing receptors, integrin β7 and CCR9, is not always linked, but instead depends on lymphocyte differentiation and the location of the effector sites infiltrated by these cells.[25,26]

Expression of MAdCAM-1 is essential for trafficking of integrin $\alpha 4\beta 7^+$ lymphocytes into the LP, while the expression of E-cadherin on the epithelium is essential for the retention of integrin $\alpha E\beta 7^+$ lymphocytes. Inflammation of the mucosa enhances MAdCAM-1 expression on the endothelial cells of post-capillary venules in the genital tract,[8] and inflammatory changes are often observed in CIN when compared with normal cervical mucosa.[27,28] Trimble et al.[9] reported that MAdCAM-1 expression correlates with non-specific $CD8^+$ LPL infiltration of the LP and CIN regression. In our sampled IELs, there was no association between $CD8^+$ cells and CIN regression. Studies have also demonstrated that oncoproteins from high-risk HPV subtypes downregulate E-cadherin expression in CIN lesions and that this downregulation is closely associated with disease progression.[29–31] E-cadherin plays an important role in the maintenance of normal adhesion in epithelial sites and its loss is associated with poor prognosis for many tumors other than CIN.[32] The downregulation of E-cadherin may interfere with the retention of integrin $\alpha E\beta 7^+$ T cells in CIN lesions, and our results suggest that IEL retention varies among patients with CIN. We have shown that populations of integrin $\alpha E\beta 7^+$ IEL in CIN lesions vary markedly among patients and that higher IEL numbers are associated with spontaneous regression of CIN. Although HPV-specific cytotoxic T lymphocyte activity was not investigated here, the accumulation of integrin $\alpha E\beta 7^+$ IEL in CIN lesions and their association with CIN regression suggests these cells, rather than non-specific $CD8^+$ T cells, may have important local effector functions in the cervical epithelium. In the present study, the adaptive immune system was focused, but the innate immune responses play equally important roles in controlling HPV infection. Daud et al.[33] has recently reported the mechanism of interference with innate immune system by HPV16, dampened toll-like receptor expression, which results in the viral persistence. The interaction of innate with adaptive immunity at the local mucosa should be investigated.

In summary, our report is the first to specifically phenotype cervical IEL in CIN lesions. Our results indicate that the presence of elevated numbers of gut-derived integrin $\alpha E\beta 7^+$ IELs in specimens gathered from patients with CIN using a cervical cytobrush may represent a possible biomarker for CIN regression. Sampling of cervical IEL using this methodology is relatively non-invasive and techni-

cally easier than the isolation of cervical LPL from tissue biopsies. Future investigations using our sampling methods will focus on HPV-specific cell-mediated immune responses by cervical IELs isolated from patients with CIN. These and related investigations should improve our understanding of cervical mucosal immunity and hasten the development of a therapeutic HPV vaccine.

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CASE REPORT

Interstitial pneumonitis induced by pegylated liposomal doxorubicin in a patient with recurrent ovarian cancer

Kanako Inaba · Takahide Arimoto · Mari Hoya · Kei Kawana · Shunsuke Nakagawa · Shiro Kozuma · Yuji Taketani

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Abstract Interstitial pneumonitis after treatment with pegylated liposomal doxorubicin (PLD) has been rarely reported. We describe herein a case of interstitial pneumonitis in a 49-year-old woman with relapsed ovarian carcinoma treated with PLD. Twenty-five days after the second administration of PLD, she presented with fever and dry cough, and chest CT scans revealed bilateral interstitial infiltrates and ground-glass opacities. She was diagnosed to have interstitial pneumonitis induced by PLD. Steroid therapy improved her symptoms.

Keywords Interstitial pneumonitis · Pegylated liposomal doxorubicin · Drug induced · Japanese · Ovarian cancer

Introduction

Pegylated liposomal doxorubicin (PLD) is an active drug in recurrent ovarian cancer as demonstrated in trials in the second-line chemotherapy [1–3]. It has been designed to enhance the efficacy and to reduce the toxicities of doxorubicin such as cardiotoxicity, hematologic toxicity, and alopecia by using a unique delivery system: a polyethylene glycol coat [4, 5]. Whereas hand-foot syndrome and planter palmar erythema are widely recognized as adverse effects of PLD, few cases of interstitial pneumonitis after treatment with PLD have been reported. Here, we describe a case of interstitial pneumonitis induced by PLD.

K. Inaba · T. Arimoto (✉) · M. Hoya · K. Kawana · S. Nakagawa · S. Kozuma · Y. Taketani
Department of Obstetrics and Gynecology, Faculty of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan
e-mail: tarimoto-tky@umin.ac.jp

Case report

A 48-year-old woman (gravida 4, para 3) with recurrent ovarian cancer was started on third-line chemotherapy with PLD (50 mg/m^2/4 weeks). She was initially diagnosed in February 2009 and underwent complete debulking surgery for a stage IIIC serous ovarian adenocarcinoma. Postoperatively, she received adjuvant chemotherapy with six cycles of paclitaxel (175 mg/m^2) and carboplatin (AUC 6). Four months later, because of peritoneum dissemination and elevation of CA125, she was treated with weekly CPT-11 (95 mg/m^2/week) with progressive disease after four cycles. In April 2010, PLD was given under her excellent performance status.

Twenty-three days after the first administration of PLD, she developed a fever from which she recovered without any treatment. However, 25 days after the second administration of PLD, she presented to our hospital with fever, chill, dry cough, and dyspnea (grade 3 according to Common terminology criteria for adverse events, version 4.0). Physical examination was remarkable for bilateral fine crackles at the lung bases. A chest X-ray and chest CT scans revealed bilateral interstitial infiltrates and ground-glass opacities, though chest CT scans performed before PLD therapy showed clear lung field (Fig. 1a, b). Oxygen saturation by pulse oximetry was 89% on room air and arterial blood gas analysis showed hypoxia (FiO_2 0.32, PaO_2 90.5 mmHg, alveolar-arterial oxygen gradient 94.9 mmHg). Laboratory analysis revealed white blood cells of 2,500/μl with 78% neutrophils, lactate dehydrogenase of 347 IU/l, C-reactive protein of 14.32 mg/dl, and Krebs von den Lungen-6 (KL-6) of 227 U/ml.

Her clinical course and laboratory data indicated that she has interstitial pneumonitis probably induced by PLD. She had not received granulocyte colony-stimulating

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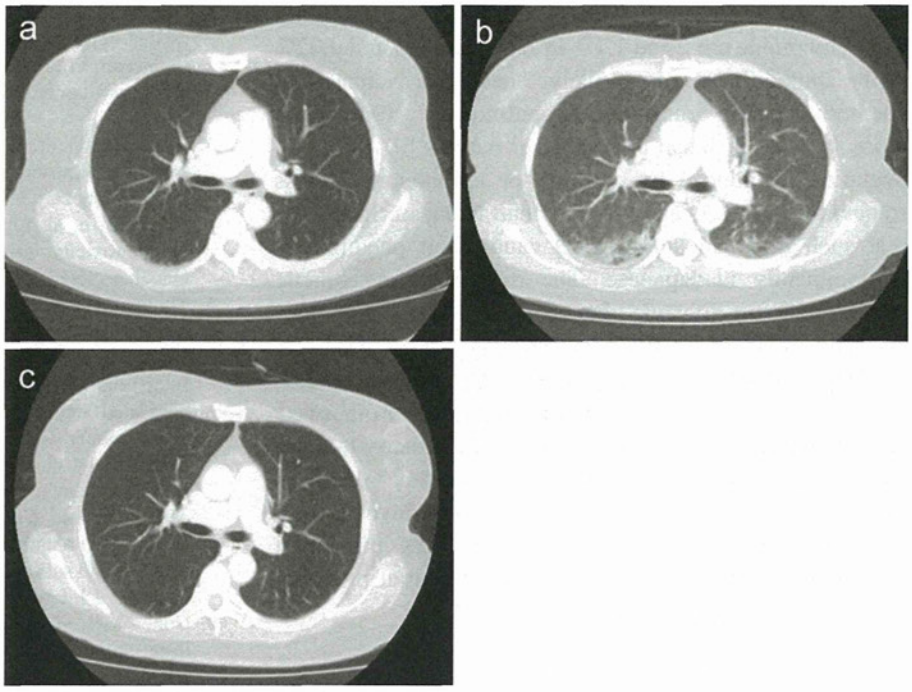

Fig. 1 **a** Chest computed tomography (CT) scan before PLD therapy showed clear lung field. **b** Twenty-six days after second administration of PLD, CT revealed bilateral interstitial infiltrates and ground-glass opacities. **c** Two months after steroid therapy, CT showed significant improvement

factor, which could induce interstitial pneumonitis. In addition to PLD, she received ascorbic acid, pyridoxal phosphate hydrate, rebamipide, and brotizolam. As they were all unlikely to induce interstitial pneumonitis, administration of these drugs except PLD was continued. The patient was treated with intravenous methylprednisolone 500 mg/day for 3 days. Azithromycin 1,000 mg per os and intravenous cefepime 4 g/day were administrated until all examinations of infection proved to be negative, including blood culture, β-D-glucan, influenza antigen detection, urinary pneumococcal antigen test, Chlamydia IgA/IgG, candida antibody assays, and galactomannan antigen of aspergillosis.

After the steroid pulse therapy, symptoms resolved promptly and lung function tests improved remarkably. Two months after the diagnosis of interstitial pneumonitis, a chest CT scan showed significant improvement (Fig. 1c). PLD was discontinued and her chemotherapy regimen was changed to docetaxel (70 mg/m^2). She has not shown any respiratory symptoms after cessation of PLD. Currently, she is alive with disease 24 months after the surgery and undergoing fifth-line chemotherapy.

Discussion

Pegylated liposomal doxorubicin is a reformulated version of doxorubicin, which takes the active agent doxorubicin and places it into a phospholipid bilayer called a liposome and another outer layer of methoxypolyethylene glycol. This coating allows PLD to evade detection and destruction by the immune system and to remain longer in the blood circulation.

PLD has a different toxicity profile compared with free doxorubicin. Though cumulative cardiac toxicities are unique to free doxorubicin, cardiac toxicities associated with PLD are rarely reported. Toxicities relatively unique to PLD are hand-foot syndrome or plantar palmar erythema (PPE), which are rarely reported with free doxorubicin.

It is reported that lung toxicity induced by doxorubicin is rare. Several cases of interstitial pneumonitis associated with doxorubicin or PLD have been described [6, 7]. It was unclear whether the lung toxicities were directly attributable to doxorubicin in published case reports, because all patients were concurrently receiving other agents, mostly antineoplastic drugs, which were also implicated in causing lung toxicity.

In our case, though the symptoms were initially severe, discontinuation of PLD and steroid therapy immediately resolved them. Serum KL-6 levels have been reported to correlate with grade of interstitial lung disease [8]. Normal serum KL-6 level in this case might associate with her excellent clinical course.

Two possible mechanisms of drug-induced interstitial pneumonitis have been described, one of which is the direct toxicity of the drug to the pulmonary organ and the other is immunological mechanism, although the etiology of PLD-induced interstitial pneumonitis is unclear.

Drug-induced pulmonary toxicity in Japan got a great deal of attention because of pulmonary toxicity induced by molecular-targeted chemotherapeutic drugs, gefitinib and an antirheumatic drug, leflunomide. It is reported that the