Population-based surveillance of Hib invasive infections in children in British Columbia, Alberta and Ontario – 1995 to 1997

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OBJECTIVE: To assess vaccine effectiveness through enhanced disease surveillance following the change in childhood immunization programs in 1995, when all provinces and territories chose to use polyribosyl ribitol phosphate-tetanus protein (PRP-T) *Haemophilus influenzae* type b (Hib) conjugate vaccine, generally in combination with diphtheria-pertussis-tetanus inactivated polio vaccine (DPT-IPV) (as PENTA vaccine) because the protective efficacy of this regimen had not been directly measured.
DESIGN: Prospective, active, laboratory-based Hib case surveillance was implemented in British Columbia and Alberta, and enhanced, stimulated laboratory surveillance in Ontario during 1995 to 1997, centred on invasive infections in children. Case details and immunization histories were uniformly collected and centrally collated.
MAIN RESULTS: Thirty-eight Hib cases were detected, but only 12 cases arose among PENTA-eligible children, an attack rate of 0.85 cases/100,000 child-years of observation. Annual case totals declined from 20 in 1995 to seven in 1997, when only one to three cases were encountered in each province and the incidence rate in children under age five years was 0.6/100,000. Only four cases occurred after primary immunization with PENTA, a failure rate of 0.28 cases/100,000 child-years of observation. Three cases among PENTA-eligible children reflected parental refusal of infant vaccinations, accounting for 25% of cases in eligible children.
CONCLUSIONS: PRP-T conjugate vaccine was highly effective when given in combination with DPT-IPV vaccine. Provincial programs that used this regimen resulted in the near elimination of invasive Hib disease in children, but unimmunized children remain at risk.

Key Words: *Children; Epidemiology;* Haemophilus influenzae*; Infection; Prevention; Vaccine*

Surveillance des infections invasives à Hib auprès d'une population d'enfants de Colombie-Britannique, de l'Alberta et de l'Ontario – 1995 à 1997

OBJECTIF : Évaluer l'efficacité d'un vaccin par le biais d'un programme de surveillance plus sophistiqué de la maladie après le changement des programmes d'immunisation infantile en 1995, alors que toutes les provinces et tous les territoires ont choisi

voir page suivante

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d'utiliser un vaccin conjugué anti-*Hæmophilus influenzæ* de type B (Hib) à base de polyribosyl ribitol phosphate (PRP-T), généralement en association avec un vaccin anti-diphtérie, coqueluche, tétanos et polyo inactivé (DCT-polyo inactivé) (sous forme de vaccin pentavalent) parce que l'efficacité de ce schéma n'avait encore jamais été mesuré directement.

MODÈLE : Étude de surveillance prospective active des cas de Hib confirmés en laboratoire, mise sur pied en Colombie-Britannique, en Alberta et programme de surveillance plus sophistiqué en Ontario, entre 1995 et 1997, axé sur les infections invasives affectant les enfants. Les détails concernant les cas et l'histoire des immunisations ont été recueillis de façon standardisée et colligés centralement.

PRINCIPAUX RÉSULTATS : Trente-huit cas de Hib ont été dénombrés, mais 12 seulement parmi les enfants admissibles au programme PENTA, soit un taux d'attaque de 0,85 cas par 100 000 années-enfants d'observations. Le nombre total de cas annuels est passé de 20 en 1995 à 7 en 1997, alors que seulement 1 à 3 cas ont été enregistrés dans chaque province, et le taux d'incidence chez les enfants de moins de cinq ans a été de 0,6/100 000. Quatre cas seulement sont survenus après l'immunisation primaire par vaccin pentavalent, un taux d'échec de 0,28 cas/100 000 années-enfants d'observations. Trois cas parmi les enfants admissibles au programme PENTA ont été le reflet direct du refus de certains parents de faire vacciner leurs enfants, représentant 25 % des cas chez les enfants admissibles.

CONCLUSION : Le vaccin conjugué par PRP-T s'est révélé hautement efficace lorsqu'il était administré conjointement avec un vaccin DCT-polyo inactivé. Les programmes provinciaux qui ont recours à ce type de schéma ont donné lieu à une élimination quasi-complète des cas de maladie invasive au Hib chez les enfants, mais les enfants non immunisés sont restés à risque.

Haemophilus influenzae type b (Hib) was, until recently, the principal cause in children of purulent meningitis, and its sequelae of deafness and mental impairment (1,2). Hib also accounted for most cases of epiglottitis and a substantial proportion of the cases of pneumonia, bacteremia, cellulitis and septic arthritis (3,4).

The control of Hib infections through immunization has been one of the major public health advances of the past decade (5,6). Disease control was achieved with a series of increasingly effective vaccines, starting with a plain capsular polysaccharide vaccine licensed in 1986 (7). The polysaccharide vaccine, containing polyribosyl ribitol phosphate (PRP), was able to protect by eliciting bactericidal and opsonizing antibodies but could not do so in children younger than 24 months of age, the group at greatest risk of Hib infection.

In 1988, a novel PRP Hib diphtheria toxoid conjugate vaccine (PRP-D) (ProHIBit, Pasteur Mérieux Connaught, Toronto, Ontario) was licensed for use in children from 18 months of age (8,9). It was incorporated into routine childhood immunization programs in all provinces and territories except Manitoba. In 1992, the current generation of Hib conjugate vaccines was licensed in Canada (10), including PRP-tetanus (PRP-T) (Act-HIB, Pasteur Mérieux Connaught), PRP-meningococcal (PRP-OMP) (PedvaxHIB, Merck Frosst Canada Inc, Kirkland, Quebec), and CRM_{197} oligosaccharide (HbOC) (HibTITER, Wyeth-Ayerst Canada Inc, Ste-Laurent, Quebec) Hib conjugate vaccines, all of which were immunogenic enough to use in infants as young as two months of age. Each product initially found a market in one or more jurisdictions, but in 1995, all provinces and territories chose to use PRP-T vaccine, generally in combination with diphtheria-pertussis-tetanus inactivated polio vaccine (DPT-IPV) (as PENTA, Pasteur Mérieux Connaught) (11). Manitoba opted to use a combination of DPT and PRP-T with oral poliomyelitis vaccine.

When PRP-T vaccine was selected for use by all provinces and territories, evidence for its protective efficacy was indirect, based on studies of immune responses (12,13). Efficacy was assumed because responses elicited by PRP-T matched or exceeded responses elicited by other Hib vaccines with demonstrated protective efficacy. Whether protection would be reduced by combining PRP-T with DPT-IPV was uncertain because serum anti-PRP levels were somewhat lower after the combination than after the separately injected vaccines (13). Also of concern was the observation that anti-PRP levels in serum declined substantially following completion of the three-dose primary series at six months of age. By the time of the recommended booster dose at 18 months of age, anti-PRP levels were undetectable or low (less than 0.15 g/mL) in 27% to 45% of children (14,15). The possibility existed that breakthrough infections might occur during this serum antibody nadir, although the minimum antibody level required for protection has not been determined for Hib conjugate vaccines. Some experts argued that because such children responded strongly to booster immunization, evidence of persistent immunological memory, susceptibility to infection was unlikely (16).

In 1994, Pasteur Mérieux Connaught sponsored several postmarketing surveillance projects to gauge the effectiveness of PRP-T vaccine given as the PENTA combination product. The study described here involved an innovative collaboration between three provinces and the Canadian Paediatric Society/Laboratory Centre for Disease Control Immunization Monitoring Program, ACTive (IMPACT) (17). The latter had been conducting Hib case surveillance at paediatric tertiary care centres across Canada since 1992 (6). The purpose of this study was to assess program and vaccine effectiveness during the initial three years of PENTA vaccine use in a large population.

PATIENTS AND METHODS

Population-based surveillance was accomplished by enlisting all relevant hospital laboratories into surveillance networks, tailored to circumstances in each participating province.

Each province carried out surveillance from January 1, 1995 to December 31, 1997, using the same case definitions. A case was considered definite if Hib was isolated from blood, cerebrospinal fluid (CSF) or other normally sterile body fluid, or from the surface of an inflamed epiglottis. In probable cases, Hib was isolated from tracheal secretions obtained through an endotracheal tube in children with pneumonia, or PRP antigen was detected in urine or CSF, except in recently vaccinated individuals. The age range was set between birth

TABLE 1
***Haemophilus influenzae* type b cases reported in three provinces from 1995 to 1997**

Province	1995	1996	1997	Total	Average annual incidence rate*
British Columbia	7	5	1	13	0.6
Alberta	4	0	3	7	0.4
Ontario	9	6	3	18	0.3
Total	20	11	7	38	0.4

**Per 100,000 persons, aged birth to 14 years*

TABLE 2
***Haemophilus influenzae* type b (Hib) cases among children eligible for PENTA vaccination from 1995 to 1997**

Province	Birth cohort annual	Hib cases (total)	Attack rate/100,000 CYO	PENTA failures	Failure rate/100,000 CYO
British Columbia	48,880	3	1.0	1	0.3
Alberta	39,720	4	1.7	1	0.4
Ontario	146,835	5	0.6	2	0.2
Total	235,435	12	0.85	4	0.28

CYO Child-years of observation

and 14 years. A vaccine failure was defined as the onset of Hib infection 28 or more days after the completion of age-appropriate primary immunization.

Isolates were identified by hospital laboratories in a routine fashion. Smaller centres referred isolates to a regional or provincial laboratory for confirmatory testing and serotyping.

In British Columbia and Alberta, specific laboratory-based surveillance networks were set up and monitored from the IMPACT hospitals in Vancouver, Edmonton and Calgary.

In British Columbia, 38 hospitals and the provincial laboratory were included in the network, encompassing all regional hospitals and all hospitals staffed by a paediatrician or having four or more paediatric beds. IMPACT program staff at BC's Children's Hospital in Vancouver contacted laboratory representatives by fax at one to three month intervals, depending on centre size. The fax reply form asked if a case had been diagnosed since the previous contact. If so, basic case information was included to enable the IMPACT monitor to request a case summary and detailed immunization history from the attending physician. Information was abstracted using a specific case report form, a copy of which was sent to the IMPACT data centre for review and collation. All replies from laboratories were tracked to avoid discontinuity.

In Alberta, all hospital laboratories in the newly regionalized health care system were included, along with the two provincial laboratories. Northern centres were contacted by the IMPACT monitor at the Health Sciences Centre in Edmonton, southern centres by the monitor at Alberta Children's Hospital in Calgary. Surveillance was carried out as above.

In Ontario, five laboratories located in tertiary care centres carried out serotyping of *H influenzae* isolates identified at their facility. All remaining Ontario laboratories submitted isolates to the public health laboratory for confirmation of serotype. For the purpose of active Hib surveillance, private and hospital laboratories were notified and reminded of the requirement under legislation to report all cases of invasive Hib disease to the local medical officer of health, who in turn reports to the Ministry of Health. Additionally, as each case report was received at the ministry, a nurse epidemiologist based at the Ministry of Health contacted the medical officer of health, the regional and central public health laboratories, and IMPACT hospitals in Toronto and Ottawa, as appropriate, to confirm and complete data related to the case. The same case report form mentioned above was used. Each of the five self-sufficient serotyping laboratories were contacted once a year to verify that all confirmed cases of Hib had been reported centrally.

At the IMPACT data centre, reports were checked for completeness and eligibility, and then entered into an electronic database. Duplicates were identified and combined.

RESULTS

During the three-year surveillance period, approximately 706,000 children were born in the participating provinces. The period of observation of these children ranged from birth to 36 months, with a median of 18 months. Based on 1996 census data, the three provinces had approximately 1.18 million children under five years of age (at highest risk of Hib infection) and about 3.5 million children under 15 years of age, yielding a total of about 10.5 million child-years of exposure to Hib infection during the survey.

Compliance of hospitals and physicians with case reporting was excellent. In British Columbia, one medium-sized hospital declined to participate, whereas all relevant hospitals in Alberta participated. Responses of laboratories to information requests were generally timely. The system in Ontario appeared to work well, given the built-in redundancies for case notification. Key information was obtained for every reported case.

In total, 38 eligible Hib cases were reported, 35 definite and three probable (positive endotracheal culture). The latter included one case of croup with suppurative tracheitis, from which Hib was the only isolate. The male to female ratio was 24:14, with males predominating in each year. Table 1 shows the distribution of cases and average annual incidence rates per province. The annual case total declined each year to a low of seven in 1997, when only one to three cases were encountered in each province. The incidence rate of infection in 1997 per 100,000 children under five years of age (based on 1996 population census figures) was 0.4 in British Columbia, 0.4 in Ontario and 1.5 in Alberta, with an overall mean of 0.6.

TABLE 3
***Haemophilus influenzae* type b syndromes reported from 1995 to 1997**

Syndrome	Ontario	Alberta	British Columbia	Total
Meningitis	10	3	1	14
Epiglottitis	6	2	4	12
Pneumonia/tracheitis	1	2	4	7
Cellulitis	2*	0	1	3*
Other, bacteremia	0	0	3	3
Total	19*	7	13	39*

**One child had both cellulitis and meningitis*

TABLE 4
Age distribution of cases of *Haemophilus influenzae* type b at admission and three-year average age incidence rates

Age	1995	1996	1997	Total (%)	Age incidence rate*
Birth to 6 months	4	0	3	7 (18.4)	2.0
7 to 24 months	9	2	3	14 (36.8)	1.3
25 to 60 months	3	6	1	10 (26.3)	0.5
5 to 9 years	3	3	0	6 (15.8)	0.17
10 to 14 years	1	0	0	1 (2.6)	0.03
Total	20	11	7	38 (100)	0.4

**Per 100,000*

Among PENTA-eligible children (ie, those born during the survey period), 12 cases were reported, an attack rate of 0.85/100,000 child-years of observation. Provincial figures are presented in Table 2.

The presenting syndromes are indicated in Table 3. Meningitis remained the most common syndrome, present in 37% of cases. Epiglottitis was a close second, accounting for 32% of cases. There were no deaths. Sequelae of meningitis were not determined.

The age distribution of cases and age incidence of infection are summarized in Table 4. Only seven cases (18.4%) occurred in children too young to have completed primary immunization; 81.6% occurred in children under five years of age. The oldest case was in a patient 10 years of age.

Immunization history was available for each case. Twenty children (52.6%) had completed appropriate primary Hib immunization, but two were severely immunocompromised at disease onset and two had missed recommended booster immunizations. The number of cases occurring after appropriate primary immunization declined progressively, from 11 in 1995 to seven in 1996 and to two in 1997. Products associated with immunization failure included polysaccharide given at 24 months of age (one case), ProHIBit (PRP-D) at 18 months (five cases), HibTITER (HBOC) at 18 months (two cases), unnamed product at 18 months (three cases), HibTITER primary series (four cases, with booster dose missed in two instances) and ActHib (PRP-T) primary series (five cases). Eleven of 20 failures followed single-dose regimens used for toddlers, and 15 instances (75%) involved products not currently used in Canada. Of the five cases that occurred after primary immunization with PRP-T, all occurred between the ages of 12 and 15 months and four followed the use of PENTA combination vaccine (Table 2). Of the PRP-T failures, only one to two instances occurred per year and per province. The only instance in British Columbia involved a severely immunocompromised child.

The preventability of the remaining 18 cases was assessed. Seven were too young to complete primary immunization, and another had just emigrated to Canada from a country where Hib vaccination was not offered. Two infants failed to complete the primary series on schedule. In five instances, the single dose meant for toddlers had been missed by the vaccination provider when other immunizations were given. Three cases occurred among PENTA-eligible children whose parents refused immunizations, accounting for 25% of cases among eligible children.

When the case experience of the IMPACT hospitals was compared with the total number of Hib cases in the corresponding province, the two sites in Alberta accounted for 100% of cases, the one site in British Columbia for 38.5% (five of 13) and the two sites in Ontario for 22.2% (four of 18).

DISCUSSION

This survey demonstrated remarkably low levels of Hib disease activity from 1995 to 1997 in three provinces using PRP-T vaccine, although the majority of cases encountered reflect on earlier programs using other Hib vaccines and schedules. Provincial case totals declined during the survey, reaching lows of three or fewer by 1997. In 1996, no cases were detected in Alberta. The incidence rate of Hib cases in 1997 among children under five years of age, the group at greatest risk, was 0.6/100,000 in the three provinces, in striking contrast with Canadian rates in the prevaccine era of 40 to 64/100,000 for this age group (18,19). Among PENTA-eligible children, the reported rate was 0.85 cases/100,000 child-years of observation.

Surveillance of invasive Hib infections is facilitated by the severity of disease caused in children. Because cases are rarely managed outside of hospital, hospital-based case ascertainment is appropriate. Moreover, cases almost always have a positive culture of blood or other normally sterile body fluid, providing a straightforward case definition and permitting laboratory-based surveillance as an efficient means of case ascertainment. Recovery of Hib organisms is occasionally

thwarted by prior antibiotic therapy, but many such cases have detectable Hib capsular antigens in body fluids (20). Cases that were only Hib antigen-positive were reportable in this survey, but no instance was encountered. Although laboratory identification of *H influenzae* is straightforward, relatively few laboratories continue to stock antisera to identify type b organisms. In this survey, measures were in place to ensure that invasive *Haemophilus* isolates were fully identified at regional or provincial laboratories, or academic centres.

The surveillance networks in British Columbia and Alberta were designed to include all relevant hospitals. In Alberta, all hospital laboratories were included because regionalization made it feasible. In British Columbia, only the smallest community hospitals (less than four paediatric beds) were excluded, on the assumption that they would refer severely ill children to regional centres. One Vancouver area hospital did not participate but reported no cases to public health authorities during the survey period. Excellent cooperation was provided by the hospitals in responding to information requests. This was facilitated by the low frequency of cases and the limited information expected from laboratory personnel when cases were reported. Monitors faced some challenges in obtaining case details from attending physicians but were ultimately successful in every instance.

The surveillance system in Ontario, which relied on primary reporting of Hib cases from laboratories with back-up reliance on physician reporting, appeared to work as well as the systems in British Columbia and Alberta. The observed average annual incidence of infection differed no more than two-fold between Ontario and the other provinces, and the incidence rate in 1997 was identical in Ontario and British Columbia. Physicians readily cooperated with case information requests from their local medical officer of health so that all desired information was available.

In none of the provinces was it feasible to confirm case ascertainment by independent means, apart from comparison with cases routinely reported to health authorities in the western provinces and annual audits at the five IMPACT hospitals.

The very low observed disease incidence rates indicate a high level of program and vaccine effectiveness. The latter could not be calculated directly because comparable pre-immunization era data are not available and intervening programs have modified disease risk. Few cases represented program failures (ie, resulted from nonvaccination or delayed vaccination), especially among children born during the survey period, but 25% of PENTA program failures had not been vaccinated because the parents refused. The number of cases declined progressively during the survey, largely because fewer failures attributable to Hib vaccines used before 1992 were encountered. In particular, PRP-D vaccine was less effective than current vaccines (8), but recipients were no longer in the age group at substantial risk of Hib infection. The number of PRP-T-related vaccination failures was remarkably low, only one to two per year and per province, amounting to less than one failure/100,000 infants immunized (Table 2). The observed PENTA failure rate was quite uniform among the three provinces (Table 2), suggesting consistent vaccine effectiveness. Among the failures detected, all occurred before booster immunization. Cases occurring after booster immunization are rare, but two instances have been reported to date by the full IMPACT network (21).

Recent publications also support a high level of effectiveness of PRP-T vaccine. A controlled, community intervention study in the Oxford region of the United Kingdom (22) demonstrated a vaccine efficacy of nearly 100% (95% CI 80% to 100%). Only one vaccine failure was detected during 16,484 child-years at risk. Nationwide immunization of infants in The Netherlands with PRP-T reduced the case total by 94% (23). Only two vaccine failures were detected during three years of surveillance. A randomized trial of PRP-T vaccine in Gambian infants demonstrated 95% protective efficacy for all types of invasive disease after three doses (24). A large scale, postlicensure study of Chilean infants also demonstrated 90.2% protection against invasive Hib disease (95% CI 74.5% to 100%) in an intention-to-vaccinate analysis (25). Additional evidence suggests that PRP-T induces immunological memory even when there is no measurable humoral response to the primary course or the response is not sustained afterward (26).

The pattern of illness caused by Hib was similar during the survey period to the preimmunization era (1,3), with most cases occurring in young children and with meningitis and epiglottitis as the most prevalent syndromes. Compared with PENTA program participants, those who refused vaccination had a relative risk of disease of 10.8 ($P<0.005$, 95% CI 2.9 to 39.8), based on an assumption that 3% refused vaccination. These observations suggest that Hib has lost none of its virulence despite the low frequency of infection.

CONCLUSIONS

PRP-T Hib vaccine, given as the PENTA combination product with DPT-IPV, has been highly effective. Hib disease incidence after three years of using this product reached an all time low in the provinces surveyed. Failures after completion of a three-dose primary series were rare. Follow-up in this survey was limited; the long term effectiveness of PRP-T will require extended disease surveillance. With the current low incidence of disease, targeted surveillance through systems such as IMPACT will be more practical than population-based surveillance.

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BOOK REVIEWS

HIV Protocols, edited by N Michael and JH Kim (1999). Humana Press, 999 Riverview Drive, Suite 208, Totowa, New Jersey, 07512, USA. ISBN 0-89603-369-4; 421 pages; US$89.50

HIV Protocols brings together a number of basic science protocols as applied to different areas of HIV research. It has been divided into four sections, which include "Virology", "Molecular Biology", "Humoral Immunology" and "Cellular Immunology". The book is part of the *Methods in Molecular Medicine* series from Humana Press, which advertises that readers will find everything that you need in the book to carry out the procedures, especially with their 'notes' section at the end of each chapter, which lets readers in on all the trade secrets. *HIV Protocols* is a good addition to a basic science laboratory that processes various HIV clinical specimens. Graduate students or clinical fellows starting out on their careers in basic experimental science will find it most useful.

"Virology", the first section of the book, has eight chapters. The first chapter is the most important because it deals with the general method for cultivation of HIV from cells and body fluids of humans infected with HIV. Two chapters look at quantitation of HIV, which includes a general method for running a reverse transcriptase assay. A chapter on the study of viral entry into the cell introduces the use of reporter viruses instead of using live viruses. The remaining chapters in this section focus on different techniques for studying the identification of chemokine receptors, which seems a little out of touch with the earlier chapters.

The second section of the book, entitled "Molecular Biology", is made up of 18 chapters. As with the first section, this one has some chapters with general molecular techniques such as Southern blotting, Northern blotting and *in situ* polyermase chain reactions specifically designed with HIV in mind, and then moves to more specific procedures. Five chapters deal with quantitation or cloning of viral RNA or proviral DNA using both homemade and commercial assays. Included here is a chapter on cloning of full length copies of the HIV provirus. For those groups involved in epidemiological field research, the two chapters, which employ dried blood and plasma spots on filter paper to identify HIV subtypes and infection status, will be of interest. Two chapters describe methods for single-strand conformation polymorphism and sequence-specific priming to identify mutations in DNA. The remaining chapters take a sharp turn, with techniques to look at HIV promoter activity and expression and an assay to measure telomere length.

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DIAGNOSIS

Blood cultures from admission grew *Salmonella typhi*, sensitive to ampicillin and ciprofloxacin (Cipro, Bayer Healthcare Division, Toronto, Ontario). Thick and thin smears for malaria were negative. Stool and urine cultures were negative. Serology for leptospirosis, rickettsia, dengue and trichenella were negative. A throat viral culture and monospot were negative. The patient was treated with ciprofloxacin for 14 days. CPK and creatinine levels fell to normal. His hematological parameters normalized, and he stopped bleeding. He had no further complications and was discharged four weeks after admission with normal renal function.

DISCUSSION

This patient experienced many common complications of typhoid fever, such as pancytopenia, delirium, hepatitis and gastrointestinal bleeding. He also developed two rare manifestations of the disease: rhabdomyolysis and renal failure. There have only been two other reported cases of rhabdomyolysis related to *Salmonella typhi* infection (1,2). However, there have been six reported cases of rhabdomyolysis associated with *Salmonella enteritidis* foodborne gastroenteritis (3,4).

Rhabdomyolysis is a syndrome characterized by elevated serum concentrations of CPK and myoglobinuria leading to renal dysfunction (5). This entity can be precipitated by numerous factors (5). Infections are a well known but less common cause of rhabdomyolysis, and should always be considered in the differential diagnosis. Singh and Scheld (4) recently reviewed the literature and compiled a comprehensive list of infections that have been reported to cause rhabdomyolysis (4). The spectrum of infectious agents that have been implicated is broad including viruses, bacteria, parasites and fungi. Viral infections were found to be a frequent cause of rhabdomyolysis, with 59 reported cases (4). Influenza is the most common viral etiology followed by HIV infection and enteroviral infection (coxsackievirus and echovirus). Other viruses reported to have caused rhabdomyolysis include Epstein-Barr virus, varicella zoster virus, cytomegalovirus and adenovirus. Bacteria were reported to cause rhabdomyolysis in 60 cases (4). *Legionella* species are the most common bacteria followed by *Streptococcus* species, *Franciscella tularensis*, *Salmonella* species, *Staphylococcus aureus, Listeria* species and *Vibrio* species. There is a case report of rhabdomyolysis from *Herbicola lathyri* (*Enterobacter agglomerans*) contamination of hyperalimentation fluid. Pseudomonal infections and leptospirosis have also been reported to cause rhabdomyolysis (4). Finally, there have been two cases of malaria, one case of candida infection and one case of aspergillus disease associated with rhabdomyolysis (4). The proposed pathophysiological mechanisms of how infections cause rhabdomyolysis include viral or bacterial invasion of skeletal muscle and toxin generation (4).

Rhabdomyolysis can be precipitated by many conditions, including infections. Physicians should be aware of the association between infections and rhabdomyolysis to aid optimal diagnosis and management of these patients.

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BOOK REVIEWS

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"Humoral Immunology" is the third section. The first two chapters describe general methods for setting up an in-house ELISA and Western blotting or immunoblotting assays to identify anti-HIV antibodies. Two chapters on antibody-antigen interaction are not very useful unless you happen to have a FACS system or a machine to measure surface plasmon reasonance. However, there is an excellent chapter describing general methods to carry out ELISPOT assays. Two different HIV infection neutralization assays are given, and the procedure needed to collect and process human mucosal specimens, which is a bit of a repeat of an earlier chapter, is described. The final chapter describes how to collect every possible specimen from all mucosal sites in a mouse, making it very applicable to research other than with HIV.

Seven chapters make up the final section entitled "Cellular Immunology". The topics covered here include lymphocyte proliferation assays, cytotoxic T-cell assays, measurement of antibody-dependent cellular cytotoxicity and detection of apoptosis, which are all important techniques in HIV research.

Overall, this book compiles a number of general basic science protocols in the fields of virology, molecular biology and immunology as applied to the study of HIV. The thorough nature of the materials and methods section of each chapter ensures that the reader will get off to a good start when using these techniques in their own laboratory.

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