Table 1 Cuproenzymes and immunomodulation

Copper enzyme	*Function*	*Immunomodulatory role*
Ceruloplasmin	Iron oxidation	Membrane integrity
Cu-Zn Superoxide dismutase	Removes superoxide	Membrane integrity
Cytochrome *c* oxidase	ATP synthesis	Energy metabolism
Dopamine-β-mono-oxygenase	Norepinephrine synthesis	Affector molecule
Peptidylglycine-α-amidating mono-oxygenase	Vasoactive intestinal peptide synthesis	Affector molecule

Possible mechanisms

Several possible mechanisms may be operative that could explain the pathogenesis of immunologic dysfunction which accompanies copper deficiency (Table 1). These mechanisms may be direct, i.e. the result of flux-limiting changes in specific cuproenzymes, or indirect. The levels of the antioxidant enzymes, Cu-Zn superoxide dismutase and ceruloplasmin, are decreased in copper deficiency and may leave the immunoreactive cells vulnerable to the increased oxygen metabolism known to accompany an activated immune response. It is also conceivable that decreased activity of cytochrome *c* oxidase may limit ATP flux and thus impair lymphocyte and macrophage energy metabolism. The complex interaction between the immune, nervous and reproductive systems may depend on copper. For example, norepinephrine synthesis depends on dopamine-β-mono-oxygenase. Norepinephrine metabolism, known to be altered by copper deficiency, is essential for immune function. Another cuproenzyme, peptidylglycine α-amidating mono-oxygenase, may also be involved; it is responsible for post-translationally modifying a large number of neuroactive peptides, including vasoactive intestinal peptide. Barnea and colleagues have shown that the release of luteinizing hormone-releasing hormone from the median eminence area is greatly amplified in the presence of copper and prostaglandin E_2. Release of luteinizing hormone from the pituitary has a major effect on gonadal hormone release, which in turn affects the thymus. The most significant impact of altered copper status may well be the level of production of IL-2.

See also: **Antioxidant micronutrients and the immune system; Mixed lymphocyte reaction (MLR).**

Further reading

Bala S and Failla ML (1992) Copper deficiency reversibly impairs DNA synthesis in activated T lymphocytes by limiting interleukin 2 activity. *Proceedings of the National Academy of Sciences of the USA* **89**: 6794–6797.

Kelley DS, Daudu PA, Taylor PC, Mackey BE and Turnlund JR (1995) Effects of low-copper diets on human immune response. *American Journal of Clinical Nutrition* **62**: 412–416.

Lukasewycz OA and Prohaska JR (1990) The immune response in copper deficiency. In: Bendich A and Chandra RK (eds) *Micronutrients and Immune Functions* pp 147–159. New York: Annals of the New York Academy of Sciences.

Prohaska JR and Failla ML (1993) Copper and immunity. In: Klurfield DM (ed) *Human Nutrition – A Comprehensive Treatise*, vol 8: *Nutrition and Immunology*, pp 309–332. New York: Plenum Press.

Prohaska JR and Lukasewycz OA (1990) Effects of copper deficiency on the immune system. In: Phillips M, Bendich A and Tengerdy R (eds) *Antioxidant Nutrients and Immune Functions*, pp 123–143. New York: Plenum Press.

CORONAVIRUS, INFECTION AND IMMUNITY

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The family of Coronaviridae was defined around 1968, primarily by morphologic criteria. The virions are pleomorphic to spherical-shaped particles (120–160 nm diameter), which are surrounded by a fringe of club- or pear-shaped surface projections (12–24 nm long; **Figure 1**). The virus has a lipid envelope and contains a single-stranded RNA genome of positive polarity. Coronaviruses infect humans, mammals and birds. Based on molecular criteria, the toroviruses represent a second genus within this family.

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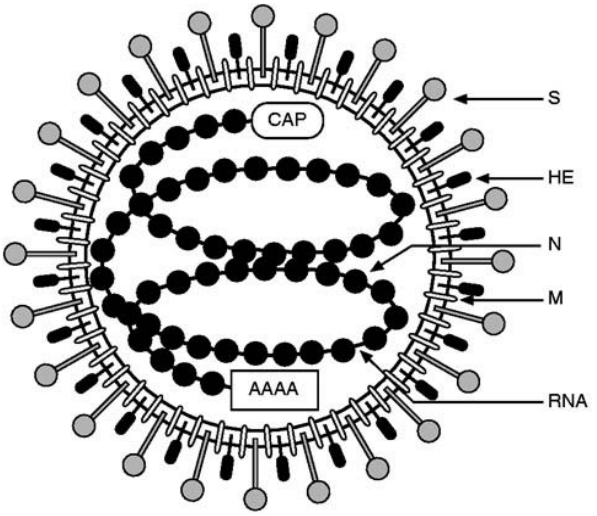

Figure 1 Coronavirus particle. The HE protein is expressed in a subset of strains only. A small membrane protein, sM, is present in the envelope of TGEV, IBV and MHV (not shown). For explanation, see text.

Furthermore, evolutionary relationships to the Arteriviridae are discussed within the concept of a 'coronavirus-like' superfamily.

Structure and replication of coronaviruses

Coronaviruses replicate in the cytoplasm and mature by budding through the Golgi apparatus and the rough endoplasmic reticulum. Their genome – a large, single-stranded molecule (size 27 000–31 500 nucleotides) – has the properties of a mRNA, namely a 5′ cap structure and a polyadenylated 3′ end. The replicase or polymerase gene 'Pol' is located at the 5′ end and contains two large open reading frames (ORF 1a and 1b, about 20 000 nucleotides) which are expressed as a fusion protein involving ribosomal frame shifting. The polymerase products generate RNA templates for replication of genomic and subgenomic mRNAs. These mRNAs contain a common 5′ leader sequence (65–100 nucleotides) that is derived from the 3′ end of the negative-sense template and is essential for a discontinuous transcription mechanism. The viral proteins are translated from a nested set of 5–7 overlapping subgenomic mRNAs with common 3′ ends. In general, only the 5′ end of each mRNA is translated into a single viral polypeptide. Certain mRNAs for nonstructural viral proteins can employ up to three ORFs via internal initiation.

All coronaviruses contain a large surface protein (S, spike, about 200 kDa), a matrix protein M (20–30 kDa) and a nucleocapsid protein N (50–60 kDa). Bovine coronavirus (BCV), human coronavirus (HCV)-OC43, hemagglutinating encephalomyelitis virus (HEV), turkey coronavirus (TCV) and some mouse hepatitis virus (MHV) strains express a hemagglutinin esterase glycoprotein termed HE (65 kDa). A small membrane protein sM (9.1–12.4 kDa) was demonstrated for transmissible gastroenteritis virus (TGEV), infectious bronchitis virus (IBV) and MHV. The typical gene order for coronaviruses is Pol-S-M-N. The position and expression of genes for HE, sM and nonstructural proteins varies between strains in a complex pattern. The helical nucleocapsid contains the genome and is surrounded by a lipid envelope (**Figure 1**). The M glycoprotein (20–30 kDa) spans this envelope three times. Peplomers (large bulbous projections) are formed by the glycoprotein S, which is cleaved in many coronaviruses to subunits of about 90 kDa (S1 and S2). The S protein induces cell fusion and binds to the host-cell receptor. A smaller fringe of spikes is formed by the HE protein, which promotes binding by interaction with 9-O-acetylated neuraminic acid.

Antigenic relationships

The majority of coronaviruses fall into three serologically distinct clusters (**Table 1**). Within each cluster, different antigenic relationships exist. The evolutionary relationships indicated by sequence data correlate by and large with this classification. Human and porcine coronaviruses are distributed in two clusters. The large number of serotypes for some coronaviruses (e.g. HCV, IBV and MHV) may result from recombination and high mutation frequencies. The S protein in particular displays a strong polymorphism. Enteric coronaviruses comprise only one serotype per group.

Diseases caused by coronaviruses

A variety of diseases of clinical and economic importance are caused by coronaviruses (**Table 1**). The majority of diseases are associated with infections of the respiratory and gastrointestinal tract. Most infections lead to acute and self-limiting diseases. Human coronaviruses are responsible for up to 30% of upper respiratory diseases, which display some periodicity in winter and spring. These viruses are difficult to isolate in tissue or organ cultures. The association of coronavirus-like particles with non-bacterial gastroenteritis is not clear.

Of veterinary importance are avian IBV, BCV, several porcine strains (e.g. TGEV, HEV and porcine respiratory coronavirus (PRCV)) and feline infectious peritonitis viruses (FIPV). Enteric coronaviruses

Table 1 Coronavirus-induced diseases

Antigenic cluster	*Virus family*	*Designation*	*Natural host*	*Typical diseases*
I	Canine coronavirus	CCV	Dog	Enteritis
	Feline enteric coronavirus	FECV	Cat	Enteritis
	Feline infectious peritonitis virus	FIPV	Cat	Peritonitis, granulomas
	Human coronavirus (229E)	HCV	Human	Respiratory disease
	Porcine epidemic diarrhea virus	PEDV	Pig	Respiratory disease
	Porcine respiratory coronavirus	PRCV	Pig	Respiratory disease
	Rabbit coronavirus	RbCV	Rabbit	Cardiomyopathy, enteritis
	Transmissible gastroenteritis virus	TGEV	Pig	Enteritis
II	Bovine coronavirus	BCV	Cow	Enteritis
	Human coronavirus (OC43)	HCV	Human	Respiratory disease
	Hemagglutinating encephalomyelitis virus	HEV	Pig	Vomiting and wasting, encephalomyelitis
	Mouse hepatitis virus	MHV	Mouse	Hepatitis, encephalomyelitis, enteritis, vasculitis
	Rat coronavirus	RCV	Rat	Respiratory disease
	Turkey coronavirus	TCV	Turkey	Enteritis
III	Infectious bronchitis virus	IBV	Chicken	Respiratory disease, nephritis, gonaditis

are only of limited pathogenicity for older animals; respiratory viruses affect adults as well. Some strains are neurotropic, others can involve many different organs. Several coronaviruses readily establish chronic infections, which can remain inapparent or modulate immune responses. In this context, MHV infections of small rodents are interesting models for investigating pathogenetic mechanisms such as demyelination or immunosuppression. Furthermore, FIPV infections in particular induce a variety of antibody-mediated immunopathologic mechanisms.

Immune responses to coronaviruses

Antibodies are generated against all structural proteins. The peplomer protein S is the major antigen for induction of neutralizing and fusion-inhibiting antibodies. Furthermore, antibodies specific for HE, N, M and sM have also been implicated in neutralization, especially in association with complement factors. Important antigenic sites of major coronavirus families are in general well defined. The bulbous S1 subunit of IBV displays high antigenic variability and induces serotype-specific neutralizing antibodies which bind to discontinuous epitopes. More conserved sites forming linear epitopes are present on the S2 subunit (stalk) and an immunodominant region was identified which is recognized by antisera against different strains. The most immunogenic sites of BCV and MHV are concentrated on the S1 subunit of the molecule. The S2 subunit of MHV also induces neutralizing and fusion-inhibiting antibodies. The neutralization of TGEV involves four major antigenic sites in the S protein, which help to differentiate members of this cluster.

BCV-specific antibodies against the HE protein are involved in neutralization. Furthermore, the M protein of MHV and TGEV induces complement-dependent neutralizing antibodies. The sM protein of TGEV appears also to be involved in neutralization. T cell responses against defined sites in the S and N protein contribute to virus elimination and can confer protection against encephalomyelitis in mice and rats. An immunodominant T cell antigenic site in the N protein of MHV helps to elicit neutralizing S protein-specific antibodies. Such T cell epitopes within the N protein have also been defined for TGEV and IBV.

Epidemiology and prevention

Coronaviruses are distributed throughout the world. Transmission does not involve vectors other than the host and occurs mostly by the oral–fecal route. The frequency of reinfection by human coronaviruses is high and vaccination may be indicated only in special cases. Administration of recombinant interferon α to volunteers prevented disease when treatment was started before infection. Mucosal immunity plays a pivotal role for the defense against respiratory and enteric coronavirus infections. The serum antibody profile does not correlate well with protection.

Licensed vaccines with attenuated live viruses exist for BCV, FIPV, IBV and TGEV. Protection against IBV is most efficient if the virus is administered to the trachea, but the continuous emergence of antigenic

variants prevents effective vaccination. Recombinant vaccinia viruses which express the S protein of IBV or FIPV can induce specific immunity; however, protection experiments with FIPV failed because antibodies elicited by defined antigenic sites of S protein accelerate the disease (antibody-mediated enhancement). Attempts to vaccinate cats with related coronaviruses were also without success.

In the mouse model, protection against encephalitis can be achieved by vaccination with a peptide which represents a surface domain of the S protein. Furthermore, immune responses against other viral proteins contribute to protection. For example, BCV can be neutralized *in vivo* by monoclonal antibodies against HE protein, and adenovirus recombinants expressing HE are capable of inducing both systemic and mucosal HE-specific immunity in cotton rats.

Enteric coronavirus infections of piglets under the age of 10 days cause high mortality that cannot be prevented by vaccination of sows. Suckling animals are naturally protected by maternal antibodies contained in milk and colostrum. This protection is mediated mainly by neutralizing S-specific IgA antibodies. Protection against TGEV can also be achieved by administration of specific serum or monoclonal antibodies. Immunization of pregnant animals with attenuated live virus is a feasible approach to control the spread of virus.

Newer experimental vaccines are mainly based on three approaches: noninfectious antigens which can be targeted to the mucosa; live vectors with enteric tropism; or internal image anti-idiotypic antibodies which mimic protective epitopes on the virus. Examples for these strategies are antigenic constructs based on peptides which represent viral immunodominant sites combined with T cell factors or bacterial components which selectively induce mucosal immunity. Furthermore, vaccines employing live bacterial vectors, adenoviruses, porcine poxviruses or genetically engineered coronaviruses are under investigation.

See also: **Influenza virus (*orthomyxovirus*), infection and immunity; Viruses, immunity to.**

Further reading

Holmes KV and Lai MMC (1995) Coronaviridae: the viruses and their replication. In: Fields BN, Knipe DM, Howley PM *et al* (eds) *Fields Virology*, pp 1075–1093. Philadelphia: Lippincott–Raven.

McIntosh K (1995) Coronaviruses. In: Fields BN, Knipe DM, Howley PM *et al* (eds) *Fields Virology*, pp 1095–1103. Philadelphia: Lippincott–Raven.

Siddell SG (ed) (1995) *The Coronaviridae*. New York: Plenum Press.

Siddell SG and Snijder EJ (1997) Coronaviruses, toroviruses and arteriviruses. In: Collier L and Mahy BWJ (eds) *Topley and Wilson's Microbiology and Microbial Infections*, 9th edn, vol 1. (*Virology*) London: Edward Arnold.

Talbot PJ and Levy GA (eds) (1995) *Corona- and Related Viruses: Current Concepts in Molecular Biology and Pathogenesis*. New York: Plenum Press.

Wege H (1995) Immunopathological aspects of coronavirus infections. *Springer Seminars in Immunopathology* 17: 133–148.

CORYNEFORM BACTERIA, INFECTION AND IMMUNITY

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Coryneform bacteria from mammalian sources can be assigned to five genera: *Brevibacterium, Corynebacterium, Dermabacter, Propionibacterium* and *Rhodococcus*. All are gram-positive, nonspore-forming bacilli, distinguished chiefly on their cell wall composition; speciation within the genera is incomplete.

Brevibacterium spp. are aerobic, nonlipid-requiring organisms which are sometimes referred to as 'large colony coryneforms'. Their pathogenic role is uncertain except in forms of tinea pedis associated with malodor caused by proteolytic destruction of the skin. Bloodstream infections are reported on rare occasions.

Corynebacterium spp. include many of the best speciated organisms; they frequently need lipid for adequate growth *in vitro* and are often microaerophils; they may be called the 'small colony coryneforms'. The localized skin disease erythrasma is caused by an overgrowth of the normal skin inhabitant *C. minutissimum*, whilst a variety of normal axillary inhabitants are associated with pronounced