Rescue with an anti-inflammatory peptide of chickens infected H5N1 avian flu.

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Chickens suffering from avian flu caused by H5N1 influenza virus are destined to die within 2 days due to a systemic inflammatory response. Since HVJ infection (1,2) and influenza virus infection (3,4) cause infected cells to activate homologous serum complement, the systemic inflammatory response elicited could be attributed to the unlimited generation of C5a anaphylatoxin of the complement system, which is a causative peptide of serious inflammation. In monkeys inoculated with a lethal dose of LPS (4 mg/kg body weight), inhibition of C5a by an inhibitory peptide termed AcPepA (5) rescued these animals from serious septic shock which would have resulted in death within a day (6). Therefore, we tested whether AcPepA could also have a beneficial effect on chickens with bird flu. On another front, enhanced production of endothelin-1 (ET-1) and the activation of mast cells (MCs) have been implicated in granulocyte sequestration (7). An endothelin receptor derived antisense homology box peptide (8) designated ETR-P1/fl was shown to antagonize endothelin A receptor (ET-A receptor) (9) and reduce such inflammatory responses as endotoxin-shock (10) and hemorrhagic shock (11), thereby suppressing histamine release in the circulation (12). Thus, we also administered ETR-P1/fl to bird flu chickens expecting suppression of a systemic inflammatory response.

Although AcPepA treatment of bird flu chickens had no beneficial effect, ETR-P1/fl administration rescued all chickens from the lethal inflammatory response (Table 1). ET-1 release is involved in histamine liberation and subsequent secondary granulocyte accumulation through tissue-specific activation of ET-A receptors. The direct effect of ET-1 induction is mainly pulmonary neutrophil activation, although MC-associated secondary changes are important in intestinal granulocyte recruitment (13). Therefore, ET-1-induced inflammation was lethal to the bird flu chickens but suppression of the ET-1 response by ETR-P1/fl rescued the infected birds from inevitable death.

Although AcPepA is a complementary peptide generated to target a portion of human C5a (aa 37-53) inhibiting its function, it might not be reactive to chicken C5a, and could fail to inhibit a cytokine storm in the virus-infected chickens. Therefore, the ineffectiveness of AcPepA on H5N1 influenza virus does not suggest that C5a inhibition could not be used as a therapeutic strategy for virus-infected patients.

On the other hand, another anti-inflammatory peptide, ETR-P1/fl, inhibited the induction of lethal symptoms following infection with H5N1 influenza virus. ETR-P1/fl could interfere with chicken endothelin activity, indicating that inhibition of the endothelin effect could be a promising therapy for treatment of patients suffering from bird flu infection resulting in serious pneumonia. Not only ETR-P1/fl, but other inhibitory agents of endothelin as well could become candidates for the therapeutic treatment of H5N1 influenza virus infection.

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Table 1

Treatment	Number of animals	Number of survivors		
		on day 1	on day 2	on day 3
H5N1 IV* only	5	5	0	0
H5N1 IV + AcPepA (2mg/kg)[$]	5	5	0	0
H5N1 IV + ETR-P1/fl (0.2mg/kg)[#]	5	5	5	5[#]

* H5N1 influenza virus (H5N1 IV) preparation at $10^{5.3}$ $TCID_{50}$ consisted of a culture supernatant of infected MDCK cells in GIT medium. Male chickens weighing 80 g (10 days of age) were intranasally inoculated with 0.2 ml of the H5N1 IV preparation on day 0.

[$]Following the virus inoculation, 0.08 ml of AcPepA (2 mg/ml in saline) were injected intramuscularly into the femoral region at a concentration of 2 mg/kg body weight. The same dose of AcPepA was injected on day 1 and day 2 as well.

[#] The two chickens used for ETR-P1/fl (0.2 mg/ml in saline) treatment were injected with 0.08 ml of the agent only on day 0, and the other 3 chickens were injected on day 1 and day 2 as well. All the ETR-P1/fl-treated chickens survived beyond 3 days.