Immunogenic properties of collagen and ovalbumin modified by chlorination

JANUSZ MARCINKIEWICZ, RAFAŁ BIEDROŃ, MONIKA MAK, MAŁGORZATA BOBEK

Department of Immunology, Jagiellonian University Medical College, Cracow, Poland

Abstract

Hypochlorite (HOCl/OCl$^-$), a product of activated neutrophils, significantly contributes to protein oxidation which occurs at a site of inflammation. Proteins modified by chlorination changed their biological activity such as enzymatic activity, resistance to proteolytic cleavage and immunogenecity. We previously reported that ovalbumin (OVA) modified with HOCl was processed and presented more efficiently than native OVA. Recently we have shown that HOCl, at the same concentrations, diminished arthritogenic and immunogenic properties of collagen type II (CII). In this study we evaluate the capacity of CII and OVA modified with HOCl to stimulate antigen-specific immune response to epitopes present on native proteins. Chlorination of OVA with 1mM HOCl results in its enhanced immunogenic properties. Chlorinated OVA more effectively stimulated OVA-specific T cell hybridoma and IgG production than native OVA. In contrast to that 3 mM HOCl completely abolished the capacity of CII to stimulate B cell response specific to native form of collagen but retained its ability to stimulate antigen specific T cells. Thus, oxidative protein modification with HOCl will result in different biological effect depending on the presence of target molecules in functionally active center of chlorinated proteins.

Key words: *neutrophils, myeloperoxidase, HOCl, antigen processing, collagen, ovalbumin*

(Centr Eur J Immunol 2003; 28 (4): 160-166)

Introduction

Inflammation is characterized by accumulation, adhesion, and activation of neutrophils and macrophages, which results in the destruction of inflamed tissue. This effect is thought to be mediated in part by the production of reactive oxygen species (ROS), a group of reactants that includes superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl) [1]. In contrast to O_2^- and H_2O_2, which do not exhibit significant reactivity with biological compounds, HOCl, a highly reactive oxidant, readily reacts with primary amines to generate long lived N-chloramines (e.g. taurine monochloramine) [2, 3]. Although N-chloramines have a lower oxidizing potential than HOCl, their much longer effective lifetime would enable them to contribute in the regulation of inflammatory response. It has been shown that taurine chloramine (TauCl), the primary neutrophil chloramines, has strong anti-inflammatory and immunoregulatory properties [4-7].

Determining the ability of HOCl to contribute in the pathogenesis of inflammatory processes associated with rheumatoid arthritis (RA) is highly dependent on determining the relevant target(s). The most likely protein target for neutrophil oxidants in RA seems to be collagen type II (CII), the major component of articular cartilage [8]. Davies et al. reported that HOCl (>1.0 mM) was required to cause direct fragmentation of CII. In addition, HOCl increased the degradation of collagen by collagenase [9, 10]. Much less is known whether the oxidative modification (chlorination) of collagen affects its immunogenic properties. Recently we have shown (manuscript in preparation) that collagen chlorination abolished its arthritogenic capacity and diminished the production of IgG antibodies specific to native collagen. On the other hand, cleavage of heat denaturated CII by neutrophil gelatinase B reveals enzyme specificity, post-translational modifications of the substrate, and the formation of remnant epitopes in rheumatoid arthritis [11]. All these data indicate that protein modification by oxidation (e.g. chlorination)

Correspondence: Janusz Marcinkiewicz, Department of Immunology, Jagiellonian University Medical College, ul. Czysta 18, 31-121 Cracow, Poland, Phone/fax number: +48 12 633 94 31, e-mail: mmmarcin@cyf-edu.kr.pl

alter immunogenic capacity of proteins and in the case of self-proteins it may result in breaking of an autotolerance and induction of autoimmunity.

The aim of the present study was to evaluate the effect of chlorination of chicken collagen type II (CII) and chicken albumin (OVA) on their capacity to induce B and T cell response specific to epitopes of native form of the antigens.

Materials and methods

Mice

Male DBA/J mice between 8-12 weeks of age, from the breeding unit of Department of Immunology, Jagiellonian University Medical College, Kraków, Poland, were used.

Protein chlorination with HOCl

Samples of chicken albumin (OVA) or chicken collagen type II (CII) (both from Sigma, St. Louis, MO) dissolved at a concentration of 2 mg/ml in 0.2 M phosphate buffer (pH 7.4), were incubated with 1, 3 or 5 mM HOCl/OCl^- at room temperature for 2 hours. To stop the reaction samples were treated with stoichiometrical amount of tiosulfate. To remove excess of free HOCl and tiosulfate, samples of chlorinated proteins were dialyzed for 24 hours in 0.2 M phosphate buffer at 4°C.

Immunization

Primary immunization: Mice were immunized intradermaly with 200 μg of either native (OVA_{NAT}, CII_{NAT}) or chlorinated (OVA_{HOCl}, CII_{HOCl}) proteins emulsified in complete Freund's adjuvant (CFA) (Sigma). The same protocol was used for OVA and CII immunization.

Booster immunization: On day 21 after the first immunization mice were injected subcutaneously with 100 μg of native protein either alone (suboptimal immunization), or in CFA. In some experiments mice received only primary immunization.

Proliferation assay

For proliferation assay, the draining lymph nodes were taken 12 days after the primary immunization. The LN cells were cultured in 96-well, flat-bottom tissue plates at a concentration $2x10^5$/well in RPMI 1640 medium (Gibco BRL, Gaithersburg, MD) supplemented with 5% FCS (Gibco BRL), 20mM HEPES, 20 mM L-glutamine, $5x10^{-5}$M 2-mercaptoethanol (all from Sigma) and antibiotics. After 72h incubation (at 37°C in 5% atmosphere of CO_2) in the presence of different concentrations of either OVA or CII, the cells were labeled with 1 μCi/well ^{3}H-thymidine for 18-20 hours and then harvested onto a glass fiber filter mat and measured in a solid scintillator by β-counter (Trilux 1 Wallac, Turku, Finland - a gift from The Wellcome Trust Foundation).

Measurement of serum IgG specific to OVA and CII by ELISA

Mice were anesthetized and bled on days 12 or 21 after primary immunization and additionally 7 days after booster immunization. Serum level of IgG antibodies against native CII or native OVA was measured using a standard ELISA assay.

Briefly, individual serum samples were stored at – 80°C until they were used for the ELISA. Microtitter plates (Corning, NY) were coated overnight with 5 μg/ml of collagen type II (acid soluble) or ovalbumin (both Sigma, Steinham, Germany) in phosphate buffered saline (PBS) at 4°C. Non specific binding was blocked with 4% bovine serum albumin (BSA) in PBS at room temperature for 1 hour. Diluted serum samples (in 1% BSA in PBS) were added and incubated for 1 hour at room temperature. The plates were then incubated with biotinylated goat anti-mouse IgG antibody (Sigma) for 45 minutes at room temperature. Horseradish peroxidase (HRP) conjugated streptavidin diluted 1:1000 in 1% BSA/PBS was added and plates were incubated for 45 minutes at room temperature. Then OPD (o-phenylenediamine dihydrochloride) (Sigma) was used as a substrate (5 mg of OPD in 10 ml of phosphate-citrate buffer, pH = 5.0) and incubated with 40 μl of 30% H_2O_2 for 30 min at room temperature. The reaction was stopped with 3M H_2SO_4. Optical density was measured at 492 nm in a plate reader (PowerWave$_X$, Bio-Tek Instruments, Winooski, VT - gift from The Foundation for Polish Science).

Activation of OVA- specific T cell hybridoma

APCs (A-20-2J – H-2^d positive B lymphoma line) ($5x10^5$/well) were preincubated in 96-well, flat-bottom tissue plates at different concentrations of native or modified OVA in RPMI 1640 medium (Gibco BRL, Gaithersburg, MD) supplemented with 5% FCS (Gibco BRL). After 2 hours of the preincubation $2x10^5$/well of OVA-specific T cell hybridoma (DO11.10 – H-2^d restricted) were added and co-cultured for additional 24 h. Next day supernatants (SN) were removed, collected and frozen in -20°C for a bioassay. The activity of interleukine-2 (IL-2) produced by the activated T cell hybridoma was measured in the SN using IL-2-dependent cytotoxic T lymphoma line cells (CTLL cells), as described previously [12]. SN from APCs co-incubated with OVA-specific T hybridoma without antigen was used as a control.

Statistical analysis

Results are expressed as mean +/- SEM. Statistical significance was determined by the Student's *t*-test and differences were regarded as significant at $p<0.05$.

Results

IL-2 release from OVA-specific T cell hybridoma stimulated with native and chlorinated OVA.

Previously we have shown an enhanced immunogenecity of OVA chlorinated (OVA_{HOCl}) with

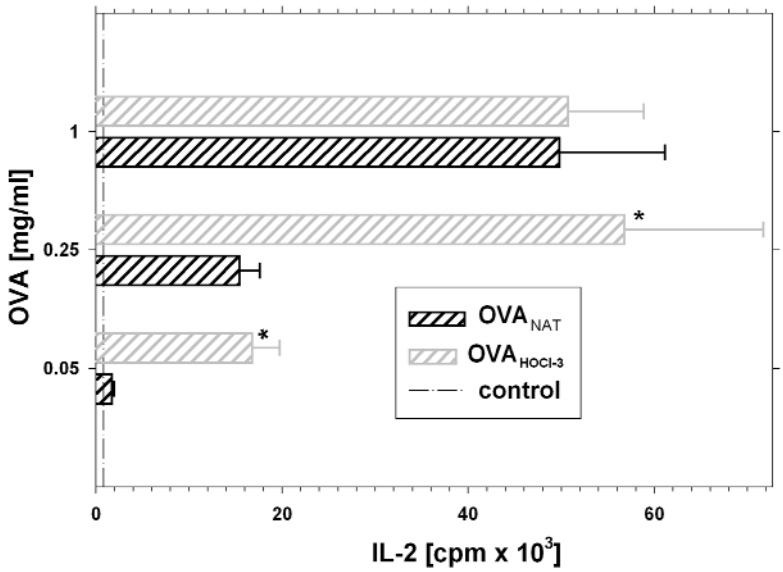

Fig. 1. IL-2 release from OVA-specific T cell hybridoma stimulated with native or chlorinated OVA.
APCs (A20-J B lymphoma line) were incubated with either native (OVA_{NAT}) or chlorinated (OVA_{HOCl}) ovalbumin. After 2 hours OVA-specific T cells (DO-11-10 T cell hybridoma) were added. After additional 24 hours supernatants were collected and the level of IL-2 produced by activated OVA-specific T cell hybridoma was measured (for details see Materials and Methods). Results are expressed as a mean +/- SEM from four independent experiments (* $p<0.01$ OVA_{NAT} vs. OVA_{HOCl})

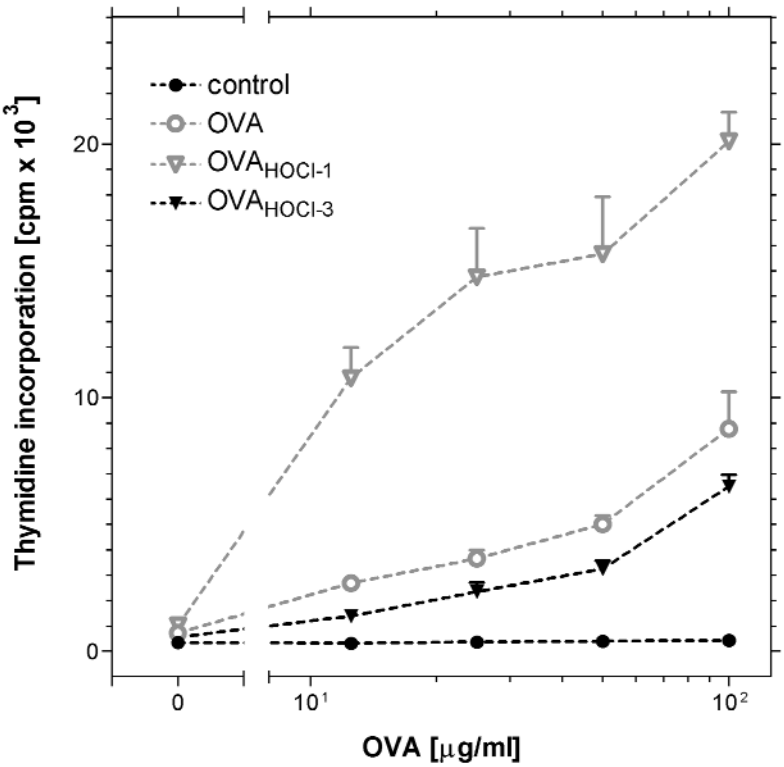

Fig. 2. *In vitro* proliferation of OVA-specific LN-cells taken from mice immunized either with native or chlorinated OVA.
Mice were immunized with either native (OVA_{NAT}) or chlorinated (OVA_{HOCl-1} = OVA chlorinated with 1 mM HOCl; OVA_{HOCl-3} = OVA chlorinated with 3 mM HOCl) ovalbumin in CFA, as described in Methods. For proliferation assay the draining lymph nodes were taken 12 days later. LN-cells were cultured at the presence of different concentrations of OVA_{NAT}. Results represent one of three independent experiments

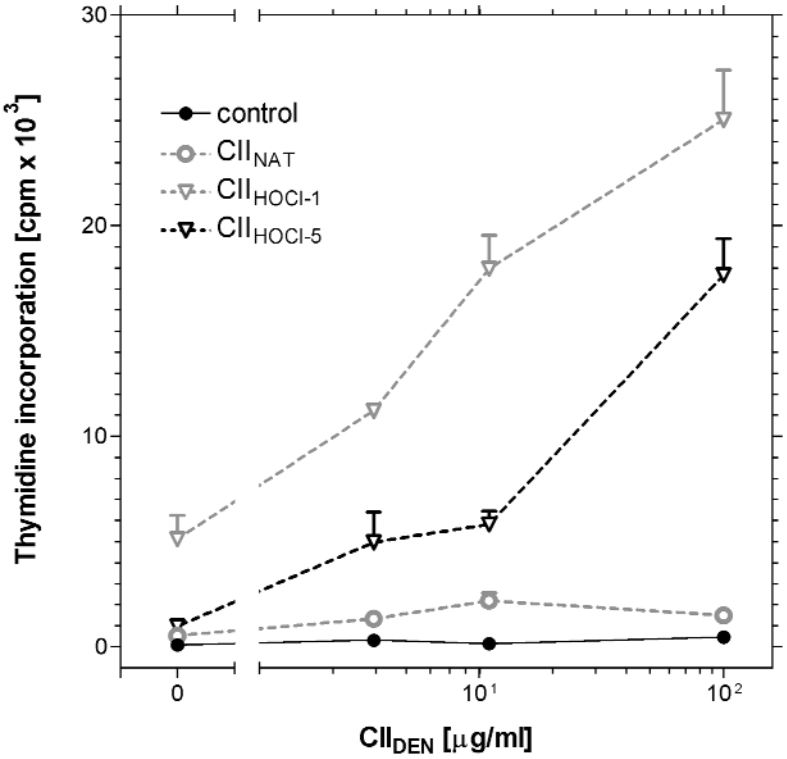

Fig. 3. *In vitro* proliferation of CII-specific LN-cells taken from mice immunized either with native or chlorinated collagen. Mice were immunized with either native (CII_{NAT}) or chlorinated (CII_{HOCl-1} = CII chlorinated with 1 mM HOCl; CII_{HOCl-5} = CII chlorinated with 5 mM HOCl) chicken collagen type II in CFA, as described in Methods. For proliferation assay the draining lymph nodes were taken 12 days later. LN-cells were cultured at the presence of different concentrations of heat denaturated collagen II (CII_{DEN}). Results represent one of five independent experiments

HOCl in the range 1.0 - 7.0 mM. Recently we have published that CII chlorinated with HOCl at concentrations above 1.0 mM lost its arthritogenic capacity. In this study to compare the influence of chlorination on OVA and CII immunogenecity, both proteins were modified with HOCl at concentrations of 1.0 and 3.0 mM.

As shown in Fig.1, OVA modified by chlorination stimulated the production of IL-2 more effectively than the native protein. The enhanced immunogenic properties of HOCl-treated OVA were observed more clearly with suboptimal doses of antigen (<0.5 mg OVA/ml).

***In vitro* proliferation of Ag-specific LN-cells taken from mice immunized either with native or chlorinated antigens.**

To determine immunogenecity of in vivo injected chlorinated antigens (OVA and CII), a proliferation assay was set up with the draining lymph node cells taken from immunized mice and restimulated in vitro with corresponding antigens. LN-cells taken from mice immunized with OVA_{HOCl-1} (OVA chlorinated with 1mM HOCl) showed stronger proliferative response than those taken from mice immunized with native OVA. No differences were found between mice immunized with native OVA and OVA_{HOCl-3} (OVA chlorinated with 3 mM HOCl) (Fig. 2.).

In contrast to that, LN-cells of mice immunized with collagen modified by HOCl (CII_{HOCl-1} and CII_{HOCl-5}) responded with a stronger proliferative response than LN-cells of mice immunized with native collagen, as shown in Fig. 3.

Antibody responses in mice immunized with HOCl-modified antigens.

Differential effect of chlorination on OVA and CII capacity to stimulate in vivo an antigen specific humoral immune response was observed. Serum IgG titer to native antigens (OVA, CII) was determined at the same timepoint after primary immunization as the proliferation assay was performed. As shown in Table1A, OVA modification with lower concentration of HOCl (OVA_{HOCl-1}) induced a twofold increment of the IgG antibody response to native, unmodified OVA as compared with IgG production after immunization with native OVA (OVA_{NAT}). On the contrary, chlorination with higher concentration of HOCl (OVA_{HOCl-3}) caused very strong decrease of IgG titers to OVA_{NAT}.

The effect of HOCl on ability of collagen to stimulate the production of IgG specific to native CII (CII_{NAT}) differ from that observed after chlorination of OVA. Chlorination of CII, decreased in a dose dependent manner, its capacity to stimulate production of IgG anti-CII_{NAT} (Table 1B). Serum IgG titer to CII_{NAT} after the immunization with CII_{HOCl-1} was 3 times lower than IgG titer after the immunization with CII_{NAT}. After immunization with CII_{HOCl-3} the level of IgG anti-CII_{-NAT} was similar to that observed in naive mice. It suggests that CII_{HOCl-3} did not stimulate antigen specific humoral response, at least to epitopes of native form of collagen. Surprisingly, primary immunization with CII_{HOCl-3} followed by suboptimal booster immunization with CII_{NAT} resulted in the production of IgG anti-CII_{NAT} (Table 2.).

Chlorination of collagen with TauCl or with HOCl in the presence of taurine did not affect capacity of collagen to induce the production of IgG specific to native antigen.

Discussion

Oxidation of proteins is a common phenomenon which occurs at a site of inflammation [13, 14]. It exerts several potential biological effects on proteins, such as alteration of enzymatic activity, alteration of susceptibility to enzymatic digestion and change in immunogenecity [15-19]. We have previously reported such biological effects of chlorination on OVA. Chlorination of OVA increased its susceptibility to proteolysis [20]. Moreover, chlorinated OVA was a stronger immunogen than the native OVA. However, the extent of chlorination was critical, as overchlorinated OVA again became a poorer immunogen [12, 21-23].

To study immunogenecity of proteins modified by chlorination we used two experimental models. Firstly, *in vitro* chlorinated chicken albumin (OVA) was incubated with APC cells and presented to OVA specific T cells. The chlorination of OVA by HOCl facilitates its processing and/or presentation by APC resulting in augmentation of IL-2 production by OVA-specific T-cell hybridoma [12]. It was also shown that chlorination facilitates proteolysis by trypsin and cathepsin D. The latter enzyme is involved in the processing of protein by APC. Secondly, *in vitro* chlorinated bovine albumin (BSA) was conjugated with trinitrophenyl (TNP) hapten. TNP-specific humoral response was tested in mice immunized with TNP-BSA conjugates composed of either native or chlorinated carrier proteins [22]. It is well known that T-dependent antigens (e.g. TNP-BSA) are recognized by both hapten-specific B cells (anti-TNP response) and carrier-specific T helper cells (anti-BSA response).We have shown that antigens (TNP-BSA) containing chlorinated carrier stimulate anti-TNP humoral response more effectively than the native TNP protein conjugate. The effect was mainly dependent on increased T cell clonal expansion [22].

Only recently we have shown (manuscript in preparation) that chlorinated chicken type II collagen lost its ability to induce collagen induced arthritis (CIA) and to

Table 1. Antibody responses to native antigens after immunization with antigens modified by HOCl

Antigen	**IgG anti native Ag**	
	[Units]	[%]
A. OVA + CFA	IgG anti OVA_{NAT} (Day 12)	
OVA_{NAT}	80±48	100
OVA_{HOCl-1}	160±72	200*
OVA_{HOCl-3}	12±6	15**
B. CII + CFA	IgG anti CII_{NAT} (Day 12)	
CII_{NAT}	190±82	100
CII_{HOCl-1}	74±62	39*
CII_{HOCl-3}	≤4	–
$CII_{TauCl-3}$	173±71	91
Naive	<4	–

**p<0.05; **p<0.001 in comparison native antigen.*
Results are reported using arbitrary defined units [1U = IgG titer 1/100] and are expressed as a mean ± SEM from 5 independent experiments. Each experimental group consist of 6-10 mice
Ag_{NAT} - native protein
Ag_{HOCl-1} – protein chlorinated with 1 mM HOCl
Ag_{HOCl-3} – protein chlorinated with 3 mM HOCl
$Ag_{TauCl-3}$ – protein treated with 3 mM TauCl

trigger the generation of IgG antibodies specific to native collagen. At the same experimental conditions tolerogenic properties of chlorinated collagen was retained. It may suggest that both, arthritogenic and immunodominant B cell epitopes of native protein were destroyed by chlorination, while some T cell epitopes were not affected.

In this study, using the same experimental conditions to modify proteins by HOCl, we compared the effect of chlorination on immunogenecity of CII and ovalbumin OVA.

Our present results confirm previous observations and demonstrate that HOCl at concentration ranging from 1.0 - 3.0 mM may enhance capacity of OVA to stimulate OVA-specific T cells. It indicates that chlorination does not affect $OVA_{323-339}$ determinant, which is recognized by DO11-10 cells, an OVA specific T cell hybridoma [12]. On the other hand, IgG production specific to B cell epitopes expressed on native OVA was not altered by 3 mM HOCl.

The effect of chlorination of CII was different from that observed for chlorination of OVA. Chlorination in a dose dependent manner decreased CII capacity to induce B cell immune response specific to native form of collagen. This effect correlated with the structural changes of collagen observed during chlorination [24]. Mice immunized with collagen modified by 3 mM HOCl, did not produce IgG antibodies specific to CII_{NAT}. It may suggest that HOCl at the concentration 3mM, in which fragmentation of collagen was observed [24], destroys B cell epitopes of native protein. Surprisingly, these mice after suboptimal booster immunization with CII produce IgG anti-CII_{NAT} antibodies. It indicates, that chlorinated collagen retained some native epitopes and during primary immunization generated T helper memory cells specific to carrier epitop(s) present on native form of collagen. This clonal expansion of T helper cells results in activation of B cells to produce antibodies against antigen used in the booster immunization. These results, together with the results which showed increased lymph node cells proliferation after immunization with chlorinated collagen suggest that chlorination preferentially enhances T cell dependent antigen-specific immune response. It may be explained by the fact that T cell epitopes are more resistant to oxidative modification than B cell epitopes [25]. Further studies are necessary to explain the effect of HOCl on structure of different proteins (see differences in immunogenecity of chlorinated OVA and CII). Primary chlorination affecting chloramine-type derivative formation, the secondary chlorination producing stable chlorotyrosine residues, chloramines decomposition products with carbonyl group formation and generation of reactive aldehydes should be taken into consideration [18].

Formation of these chlorine moieties in the polypeptide chain containing antigen epitopes will result in an altered antigen immunogenecity.

Conclusion

In conclusion, our present and previous studies, concerning the role of MPO-halide system in modification of the immune response showed that oxidative modification of proteins by HOCl may alter their biological functions including their resistance to proteolytic cleavage, immunogenecity and pathogenecity. Chlorination will affect distinct proteins in different ways depending on the presence of target functional groups ($-NH_2$, -SH, -S-S, >C=O, tyrosine, tryptophan) [20, 21]. More generally, our

Table 2. Priming with chlorinated collagen enhances IgG production to CII_{NAT} after booster immunization with suboptimal doses of native collagen

Ag administration		IgG anti CIINAT [Units]	
Priming* **(0)**	**Boost**** **(21)**	**Day (21)**	**Day (21+7)**
∅	C_{NAT}	–	<4
CII_{NAT}	∅	512	1024
CII_{NAT}	C_{NAT}	512	1024
CII_{HOCl-1}	∅	64	64
CII_{HOCl-1}	C_{NAT}	64	1024
CII_{HOCl-3}	∅	<4	<4
CII_{HOCl-3}	C_{NAT}	<4	256

Results represent one of five independent experiments. Each experimental group consist of 6-10 mice.

** intradermal immunization with 200 μg CII in CFA*

*** subcutaneous immunization with 100 μg CII without adjuvant*

CII_{NAT} – native collagen

CII_{HOCl-1} – collagen chlorinated with 1 mM HOCl

CII_{HOCl-3} – collagen chlorinated with 3 mM HOCl

study provides further evidence for a role of neutrophils in modulation of adaptive immunity. As neutrophils are the first cells at a site of acute inflammation, the products they release (HOCl, TauCl) have the capacity to influence antigen immunogenecity, APC function and the subsequent function of effector T cells. Further studies are necessary to estimate the role of chlorination of proteins (tagging of autoantigens) in etiopathogenesis of autoimmune diseases such as rheumatoid arthritis.

Acknowledgments

This work was supported by grant from the Committee of Scientific Research (Warsaw, Poland) Grant No. 4PO 5B 01018. T cell hybridoma activation by chlorinated ovalbumin was partially made in the Department of Immunology University College London as a part of collaboration with Prof. B. Chain.

References

1. Hampton MB, Kettle AJ, Winterbourn CC (1998): Inside the neutrophil phagosome: oxidants, myeloperoxidase and bacteria killing. Blood 92: 3007-3017.
2. Weiss SJ, Klein R, Slivka A, Wei M (1982): Chlorination of taurine by human neutrophils. Evidence for hypochlorous acid generation. J Clin Invest 70: 598-607.
3. Zgliczyński JM, Stelmaszyńska T (1975): Chlorinating ability of human phagocytosing leukocytes. Eur J Biochem 56: 157-162.
4. Marcinkiewicz J (1997): Neutrophils chloramines - missing link between innate and acquired immunity. Immunol Today 18: 577-581.
5. Marcinkiewicz J, Grabowska A, Chain BM (1998): Modulation of antigen-specific T-cell activation in vitro by taurine chloramine. Immunology 94: 325-330.
6. Park E, Schuller-Levis G, Quinn MR (1995): Taurine chloramine inhibits production of nitric oxide and TNF-alpha in activated RAW 264.7 cells by mechanisms that involve transcriptional and translational events. J Immunol 154: 4778-4784.
7. Kontny E, Grabowska A, Kowalczewski J, et al. (1999): Taurine chloramine inhibition of cell proliferation and cytokine production by rheumatoid arthritis fibroblast-like synoviocytes. Arthritis Rheum 42: 2552-2560.
8. Trentham DE (1982): Collagen arthritis as a relevant model for rheumatoid arthritis. Arthritis Rheum 25: 911-916.
9. Davies JM, Horwitz DA, Davies KJ (1994): Inhibition of collagenase activity by N-chlorotaurine, a product of activated neutrophils. Arthritis Rheum 37: 424-427.
10. Davies JM, Horwitz DA, Davies KJ (1993): Potential roles of hypochlorous acid and N-chloroamines in collagen breakdown by phagocytic cells in synovitis. Free Radic Biol Med 15: 637-643.
11. Van den Steen PE, Proost P, Grillet B, et al. (2002): Cleavage of denatured natural collagen type II by neutrophil gelatinase B reveals enzyme specificity, post-translational modifications in the substrate, and the formation of remnant epitopes in rheumatoid arthritis. FASEB J 16: 379-389.
12. Marcinkiewicz J, Chain BM, Olszowska E, et al. (1991): Enhancement of immunogenic properties of ovalbumin as a result of its chlorination. Int J Biochem 23: 1393-1395.
13. Naskalski JW, Marcinkiewicz J, Drożdż R (2002): Myeloperoxidase-mediated protein oxidation: its possible biological functions. Clin Chem Lab Med 40: 463-468.
14. Anderson MM, Requena JR, Crowley JR, et al. (1999): The myeloperoxidase system of human phagocytes generates Nepsilon-(carboxymethyl)lysine on proteins: a mechanism for producing advanced glycation end products at sites of inflammation. J Clin Invest 104: 103-113.
15. Allison ME, Fearon DT (2000): Enhanced immunogenicity of aldehyde-bearing antigens: a possible link between innate and adaptive immunity. Eur J Immunol 30: 2881-2887.
16. Claesson R, Karlsson M, Zhang Y, Carlsson J (1996): Relative role of chloramines, hypochlorous acid, and proteases in the activation of human polymorphonuclear leukocyte collagenase. J Leukoc Biol 60: 598-602.
17. Hazen SL, Hsu FF, d'Avignon A, Heinecke JW (1998): Human neutrophils employ myeloperoxidase to convert alpha-amino acids to a battery of reactive aldehydes: a pathway for aldehyde generation at sites of inflammation. Biochemistry 37: 6864-6873.
18. Hawkins CL, Davies MJ (1999): Hypochlorite-induced oxidation of proteins in plasma: formation of chloramines and nitrogen-centred radicals and their role in protein fragmentation. Biochem J 340: 539-548.
19. Jerlich A, Pitt AR, Schaur RJ, Spickett CM (2000): Pathways of phospholipid oxidation by HOCl in human LDL detected by LC-MS. Free Radic Biol Med 28: 673-682.
20. Olszowska E, Olszowski S, Zgliczyński JM, Stelmaszyńska T (1989): Enhancement of proteinase-mediated degradation of proteins modified by chlorination. Int J Biochem 21: 799-805.
21. Olszowski S, Olszowska E, Stelmaszyńska T, et al. (1996): Oxidative modification of ovalbumin. Acta Biochim Polon 43: 661-672.
22. Marcinkiewicz J, Olszowska E, Olszowski S, Zgliczyński JM (1992): Enhancement of trinitrophenyl-specific humoral response to TNP proteins as the result of carrier chlorination. Immunology 76: 385-388.
23. Marcinkiewicz J, Nowak B, Grabowska A, et al. (1999): Regulation of murine dendritic cell functions in vitro by taurine chloramine, a major product of the neutrophil myeloperoxidase-halide system. Immunology 98: 371-378.
24. Olszowski S, Mak P, Olszowska E, Marcinkiewicz J (2003): Collagen type II modification by hypochlorite. Acta Bioch Pol 50: 471-479.
25. Myers LK, Seyer JM, Stuart JM, et al. (1993): T cell epitopes of type II collagen that regulate murine collagen-induced arthritis. J Immunol 151: 500-505.