Thermo-responsive poly-*N*-isopropylacrylamide as an adjuvant in experimental rheumatoid arthritis

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Statement of Purpose: Rheumatoid arthritis (RA) is an autoimmune disease mediated by the concerted action of the innate and acquired immune systems in which inflammation is associated with progressive destruction of the extracellular matrices of bone and cartilage. To dissect disease pathways and genes modulating arthritis development, several animal models are used [1]. Most of the induced models of arthritis (both chronic and acute) use an adjuvant for inducing/enhancing the disease development. Most commonly used adjuvants often tend to deviate the ensuing immune responses [2], thereby precluding our understanding of actual immune responses to self-proteins. Moreover these adjuvants also are associated with toxicity problems. In this context, we tested adjuvant property of thermo-responsive biocompatible and biocompatible polymer, poly-*N*-isopropylacrylamide (PNiPAAm).

Methods: PNiPAAm was synthesized through free radical polymerization and characterized *in vitro* as well as *in vivo*. The cloud point of PNiPAAm (0.1%, w/v) was determined by abrupt change in the absorbance of polymer solution at 450 nm, using a HEλIOS α spectrophotometer [3]. The molecular weight of polymer was determined by gel permeation chromatography (Waters, US) and adjuvancity of was evaluated in 7-8 weeks old B10.RIII mice. Immunostaining was performed to detect changes in the conformation of collagen II (CII) after mixing it with PNiPAAm. The amounts of total anti-CII IgG were determined through quantitative ELISA. Scoring of animals was done using a scoring system based on the number of inflamed joints in each paw.

Results: High molecular weight (120kDa) PNiPAAm was synthesized through radical polymerization with cloud point ~32°C. The polymer precipitates *in situ* and releases slowly the antigen, CII at 37°C over period of time (Fig.1A). The released CII retained its native conformation (Fig. 1B). The PNiPAAm showed adjuvant activity for induction of arthritis and it was that there was 38% disease incidence score in mice injected with PNiPAAm-CII injection compared to CFA-CII (68%) (Fig.1C). Moreover, maximum mean arthritis score of PNiPAAm-CII was 22±5 in comparison to 23±3 with CFA-CII. Subsequently, we observed good anti-CII response in mice immunized with PNiPAAm–CII and after booster injection (21 days) it was comparable to CFA-CII (Fig.1D). Both PNiPAAm-CII and CFA-CII immunized group showed a significant inflammation in the articular joints. Histological features of the joints of the animals that had clinical disease were assessed 50 days after immunization. Massive infiltration of inflammatory cells accompanied by joint and cartilage erosions were observed (Fig.1F) in comparison to normal joint (Fig. 1E).

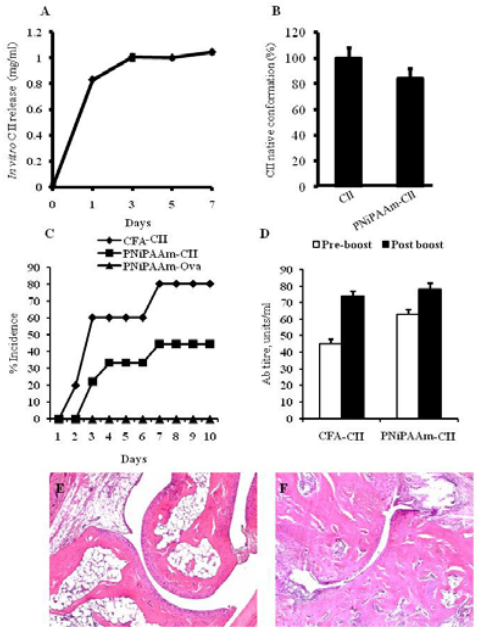

Fig. 1. *In vitro* characterization and adjuvant effect of PNiPAAm, A; In vitro released profile of CII after mixing with PNiPAAm at 37°C, B; Native conformation of released CII, C; Percent incidence of arthritis injected with CII with CFA (n= 19) or PNiPAAm (n= 48), D; Anti-CII antibody response, Hematoxylin and eosin staining of hind paw normal joint E, and disease joint F. Magnification x 20. Error bars indicate ± SEM. n denotes number of mice in each group.

Conclusions: The synthesized thermo-responsive PNiPAAm showed the temperature dependent phase transition. The CII mixed with PNiPAAm precipitated inside the body (gelation) and released antigen slowly for a longer period. The CII did not change its native conformation after mixing with polymer and induced significant anti-CII antibody response and arthritis. Therefore, biodegradable thermo-responsive polymers can be used as an adjuvant to study not only autoimmuity but also arthritis development.

References

1. Monach PA. Adv Immunol 2004;82:217-248.
2. Reed SG. Trends Immunol 2009;30:23-32.
3. Shakya AK. Enzyme Microb Tech 2010;47:277-282.